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RESEARCH, DEVELOPMENT & ENGINEERING CENTER

U.S. ARMY CHEMICAL AND BIOLOGICAL DEFENSE COMMAND

ERDEC-TR-176

ERDEC CONTRIBUTION TO THE 1993 INTERNATIONAL TREATY VERIFICATION ROUND ROBIN EXERCISE 4

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In March 1993, the U.S. Army Edgewood Research, Development and Engineering Center along with 16 other laboratories, participated in the 4th International Treaty Verification Round Robin Exercise. The objective of the exercise was to evaluate the current recommended operating procedures for the analysis of scheduled compounds in soil and water matrices. Eleven spiked samples and blanks resulting from three different types of soil and one source of water were received. Analytical methods used to analyze the samples were GC/FID/FPD, GC/MS (EI and methane CI), NMR (^{1}H, ^{13}C, and ^{31}P), and HPLC/ion chromatography. Four schedule 2 degradation products of VX and BZ (methylphosphonic acid, 2-diisopropylamino-ethanol, 3-quinuclidinol, and benzilic acid) were unambiguously identified and quantitated in the samples. This report summarizes the analytical methodology used in this round robin and the results obtained.

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PREFACE

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ERDEC CONTRIBUTION TO THE 1993 INTERNATIONAL TREATY VERIFICATION ROUND ROBIN EXERCISE IV

1. INTRODUCTION

In March 1993, the U.S. Army Edgewood Research, Development and Engineering Center (ERDEC), Research and Technology Directorate, participated in the 4th International Inter-Laboratory Comparison (Round Robin) Test for the Verification of Chemical Disarmament. The objective of the exercise was to test the recommended operating procedures (ROP's) for the analysis of scheduled compounds in soil and water matrices as outlined in the Round Robin Bluebook F.3.[1] The sixteen laboratories participating in the test were Australia, Canada, China, the Czech Republic, Finland, France, Germany, the Netherlands, Norway, Russia (two laboratories), Switzerland, Sweden, the United Kingdom, and ERDEC and the Lawrence Livermore National Laboratory (LLNL) from the United States. Lawrence Livermore prepared the samples. Finland served as the coordinating laboratory.

Eleven spiked samples and blanks resulting from three different types of soil and one source of water were received 16 February 1993. The only information provided was that no actual Schedule 1 compounds were added to the samples. Each laboratory was asked to identify and quantitate all scheduled compounds and related degradation products present, with a starting date of 1 March 1993. Participants were asked to follow the ROP's as closely as possible to validate existing methods and were also encouraged to apply additional methods to further improve existing ROP's. Sample preparation was started 2 March 1993 and analyses were completed 26 March 1993. Analytical methods used to analyze the samples were GC/FID/FPD, GC/MS (EI and methane CI), NMR (^{1}H, ^{13}C and ^{31}P), and HPLC/ion chromatography. A description of the samples and the rationale behind the selection of the spiking materials and the sample preparation methods as provided by LLNL is given in Appendix A.

This report summarizes the sample preparation, the analytical approach and methods used, chain of custody and QA/QC procedures, quantitation methods, and results obtained by each analytical method using both the recommended operating procedures and alternative procedures.

[1] INTERNATIONAL INTERLABORATORY COMPARISON (ROUND-ROBIN) TEST FOR THE VERIFICATION OF CHEMICAL DISARMAMENT F.3 Testing of Procedures on Simulated Military Facility Samples, The Ministry of Foreign Affairs of Finland (Marjatta Rautio, editor), Helsinki 1992.

2. EXPERIMENTAL PROCEDURES

2.1 Receipt and Storage of Samples

Samples were received from LLNL on 16 February 1993 by the QA/QC coordinator for Research and Technology Directorate. Inspection upon receipt revealed that the containers were in excellent condition. Samples were stored at 4 °C in a locked refrigerator, with access only by the QA/QC Coordinator, until 1 March when 20 g of each soil sample and 20 mL of each water sample were transferred via DA Form 4137 to the sample custodian for the Sample Analysis Team. Samples were stored in a secured refrigerator at 6 °C in the sample analysis laboratory, with access only by the sample custodian, except during sample preparation and analysis. Additional samples were obtained from the QA/QC Coordinator as required.

Overall, 11 samples resulting from three different types of soil and one source of water were received and were identified as follows:

SA-15	Spiked soil sample
SAB-15	Soil blank for SA-15
SB-15	Spiked soil sample
SBB-15	Soil blank for SB-15
SN-15	Spiked soil sample
SNB-15	Soil blank for SN-15
W-15	Spiked water sample
WB-15	Water blank for W-15
KEW-14	Water passing through C-18 cartridge
K-15	C-18 cartridge associated with KEW-15
KB-15	Blank C-18 cartridge

It was noted that the KEW sample was labelled KEW-14 rather than KEW-15, resulting from either a labelling or shipping error. The error was reported to Lawrence Livermore on 2 March 1993. 50 g of each soil sample and 50 mL of each water sample were provided. Samples were reported to contain Schedule 1 degradation products but no Schedule 1 material. The two water samples were identified as coming from the same source but received different treatment in order to evaluate the feasibility of using a C-18 solid phase extraction cartridge for extracting water in the field. One water sample was preserved with 0.5 mL methylene chloride per liter of water and shipped as is (W-15). The second water sample was passed through a C-18 cartridge, and both the cartridge and water shipped with no preservative (K-15 and KEW-14, respectively).

2.2 Chain of Custody Procedures

Round Robin sample transfers were documented using DA Form 4137 chain of custody procedures as described in the enclosed IOP No. 001.00, "Chain of Custody, Laboratory Sample Distribution, and Sample Documentation for Round Robin Test #4", Appendix B.

2.3 Sample Preparation

Soil and water samples were prepared as outlined in the recommended operating procedures with a slightly modified procedure for NMR analysis. The approach taken was to have two separate sample preparation schemes, one in deuterated solvents specifically for NMR analysis and one in non-deuterated solvents for all other methods of analysis. Measured extraction volumes and extraction scheme flow charts are included in Appendix C.

2.3.1 Preparation of Soil Samples

Soil samples were prepared as outlined in section SP5 of the Round Robin Bluebook F.3. Samples were assumed to be homogeneous. After warming to room temperature, a 10 gram sample was sequentially extracted with 2 x 10 mL methylene chloride, 2 x 10 mL HPLC grade water and 2 x 10 mL 0.5M KOH/methanol. Each extraction mixture was sonicated in ice for 10 minutes and centrifuged for 10 minutes at 2000 rpm. The methylene chloride fraction was filtered through a 0.45 μm filter, dried with anhydrous sodium sulfate and concentrated by nitrogen blowdown to 2 mL (sample A*). The water fraction was filtered through a 1.0 μm disposable filter. After transferral of 1 mL for direct analysis by HPLC (sample B*), the water fraction was evaporated to dryness by rotovap, dissolved in 2 mL methanol, sonicated for 5 minutes, and filtered through a 1.0 μm filter (sample C*). The KOH/methanol fraction was neutralized with 4M HCl, the methanol evaporated by nitrogen blowdown, and extracted with 2 x 2 mL hexane with shaking for 5 minutes. The hexane extract was filtered through a 0.45 μm filter, dried with anhydrous sodium sulfate, and concentrated to 2 mL by dry nitrogen blowdown (sample D*). 50 μg of the internal standard dibenzothiophene (DBT) was added to each fraction for quantitation. The extraction scheme for soil using non-deuterated solvents is summarized in Table C-3 and Scheme C-1.

A similar extraction scheme was used with deuterated solvents for NMR analysis. A 10 gram soil sample was extracted sequentially with 2 x 10 mL $CDCl_3$, 2 x 10 mL D_2O, and 2 x 10 mL 0.5M KOH/methanol. Extracts were sonicated in ice for 10 min (5 minutes for the D_2O extract) and centrifuged for 10 minutes at

2000 rpm. The $CDCl_3$ extract was dried with anhydrous sodium sulfate, filtered through a 0.45 μm disposable filter and concentrated to 1 mL by dry nitrogen blowdown (sample A). The D_2O extract was filtered and concentrated to 1 mL by dry nitrogen blowdown (sample B). The KOH/methanol extract was dried with anhydrous sodium sulfate, filtered through a 0.45 μm disposable filter, concentrated to dryness by dry nitrogen blowdown, and taken up in 1.5 mL CD_3OD for NMR analysis (sample C). The soil extraction scheme for NMR analysis is summarized in Table C-4 and Scheme C-2.

It should be noted that early extractions in all organic solvents resulted in a high NMR background level. The problem was traced to the disposable filters used during sample preparation by analyzing pure $CDCl_3$ solvent that was passed through the filter. Although the filters had teflon membranes, apparently there was some reaction with or extraction of the housing. Subsequent filtration of organic solvents was done using a 100% cotton plug rather than the disposable filters.

2.3.2 Preparation of Aqueous Samples

Aqueous samples for chromatographic analyses were prepared as outlined in section SP7 of the Round Robin Bluebook F.3. After transferring 1 mL for HPLC analysis (sample A*), a 9 mL water sample was sequentially extracted with 2 x 2.5 mL CH_2Cl_2 at neutral pH (as received, pH 8), acidic pH (adjusted to pH<2 with dilute HCl), and basic pH (adjusted to pH>10 with dilute KOH). Each extract was dried with anhydrous sodium sulfate, adjusted to a volume of 2 mL, and 50 μg internal standard (dibenzothiophene) added. The remaining aqueous layer was evaporated to dryness, reconstituted in 2 mL methanol, dried, and 50 μg internal standard added. The samples were designated B* (neutral extract), C* (acidic extract), D* (basic extract) and E* (residue). Extraction procedures are summarized in Table C-5 and Scheme C-3.

Aqueous samples for NMR analysis were prepared using a slightly modified procedure. Instead of organic extraction at neutral, acidic, and basic pH, only one extraction was done at neutral pH, to extract any volatile organic compounds that may be present. After transferring 1 mL to an NMR tube for direct analysis (sample A), a 9 mL sample of water was extracted with 2 x 0.75 mL $CDCl_3$. The extract was dried with anhydrous sodium sulfate and analyzed (sample B). The remaining water fraction was concentrated to dryness with dry nitrogen blowdown and the residue dissolved in 1 mL D_2O (sample C). This extraction procedure is summarized in Table C-6 and Scheme C-4.

2.3.3 Preparation of C-18 Cartridge Samples

The C-18 cartridges were eluted with acetonitrile into a 7.5 mL tared teflon-stoppered vial until 5 mL was collected gravimetrically. 1 mL aliquots were used for subsequent derivatization and analysis. C-18 cartrige extraction procedures are summarized in Table C-7 and Scheme C-5.

2.3.4 Derivatization

To obtain optimum results, samples were derivatized using both bis(trimethylsilyl)trifluoroacetamide (BSTFA) for trimethylsilylation and diazomethane for methylation. Sample volumes were adjusted to 2 mL, 50 μg of dibenzothiophene added as an internal standard, and the sample divided into 1 mL aliquots for subsequent derivatization.

Trimethylsilylation was accomplished by evaporation of the sample to dryness by either dry nitrogen blowdown or rotary evaporator and adding 0.25 mL dry THF and 0.25 mL BSTFA. The solution was heated for 20 minutes at 60 °C.

Methylation was accomplished using Diazald (N-methyl-N-nitroso-p-toluenesulfonamide) as the diazomethane generator in ether. The diazomethane solution was added to the sample at room temperature and the excess diazomethane blown off with dry nitrogen after 20 minutes.

2.4 Materials

Non-deuterated methylene chloride, methanol and hexane used in this study were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO and Milwaukee, WI) as HPLC-grade solvents. Tetrahydrofuran (THF) was obtained as Eastman White Label from Eastman Chemical Company (Rochester, NY) and dried over molecular sieves. Ethyl ether was obtained as Reagent ACS grade (Fisher Scientific Company, Pittsburgh, PA). Deuterated chloroform, $CDCl_3$ (99.8 atom % D) and deuterated water, D_2O (99.9 atom % D) were obtained from MSD Isotopes (Montreal, CA). Deuterated methanol, CD_3OD (99.8 atom % D), was obtained from Isotec, Inc (Miamisburg, OH). BSTFA was obtained from SUPELCO, Inc (Bellefonte, PA) in sealed ampoules and Diazald was obtained from Aldrich Chemical Company (Milwaukee, WI)

Authentic reference standards for all four identified Schedule 2 compounds were available in our laboratory for unambiguous identification. Methylphosphonic acid (MPA) was obtained from FMC Corporation (Baltimore, MD). 2-Diisopropyl-aminoethanol (DIAE) was synthesized and distilled in house.

3-Quinuclidinol (3-Q) was obtained from Fluka Chemical Corporation (Ronkonkoma, NY). Benzilic acid (BA) was obtained from Chem Service (Media, PA).

2.5 Instrumentation

2.5.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H, ^{13}C and ^{31}P NMR spectra were obtained using a Varian VXR-400S superconducting Fourier transform (FT) NMR spectrometer system (Varian Associates, Palo Alto, CA). Specific conditions and parameters of analysis are described in the NMR Appendix D.

2.5.2 Gas Chromatography/Mass Spectrometry (GC/MS)

Three GC/MS systems were used in this study. Electron ionization (EI) spectra were obtained using a Hewlett-Packard 5890 gas chromatograph interfaced with an HP-MSD 5970A mass selective detector (Hewlett-Packard Company, San Fernandc, CA). Methane chemical ionization (CI) spectra were obtained using a Finnigan 5100 GC/MS (Finnigan Corporation, San Jose, CA). Both EI and CI spectra were obtained using a Perkin-Elmer 8500 gas chromatograph (Perkin-Elmer Corporation, Norwalk, CT) interfaced with a Finnigan ITD 40 ion trap detector. Specific GC and MS parameters and conditions are listed in Appendix E (MS) and Appendix F (GC).

2.5.3 Gas Chromatography (GC)

Gas chromatography was used to screen for phosphorus and sulfur containing compounds. GCs used in this study include a Hewlett-Packard 5880 (FID), a Varian 6000 (FPD, P-mode) and a Perkin-Elmer 8500 (FPD, S-mode). Specific GC parameters and conditions used in this study are listed in Appendix F.

2.5.4 Ion Chromatography (IC)

Ion chromatography was used to confirm the presence of methylphosphonic acid and benzilic acid and as a screening technique for ethyl methylphosphonic acid, isopropyl methylphosphonic acid, pinacolyl methylphosphonic acid, thiodiglycol, thiodiglycol sulfoxide, and thiodiglycol sulfone. Analyses were performed using a Dionex Model DX-300 Ion Chromatograph (Dionex Corporation, Sunnyvale, CA) equipped serially with both a variable wavelength UV detector and a Dionex model PAD-1 pulsed amperometric detector operating with a platinum working electrode. Specific parameters and conditions used are delineated in Appendix G.

2.6 Analytical Approach

Although NMR is normally used as a confirmation technique, in this exercise we reversed roles and used NMR as a screening technique. Sample preparation for NMR was done first to allow time for overnight experiments. This approach worked extremely well. Not only was NMR able to unambiguously identify four Schedule 2 compounds (methylphosphonic acid, 2-diisopropyl-aminoethanol, 3-quinuclidinol and benzilic acid) by comparison to authentic standards, but we were able to obtain an estimation of the concentration levels of the spiked compounds as well. This information served as an aid in the selection of a suitable internal standard for GC/MS quantitation which did not interfere with the analytes and in the selection of the concentration of the internal standard. The addition of 50 μg of internal standard to each extract was chosen to match the anticipated concentrations of the analytes based on the NMR data. The initial identification of the compounds also led to the decision to use both diazomethane and BSTFA derivatization to identify the analytes.

Initial confirmation of the NMR assignments was obtained by placing 1 μL of the water sample W-15 on a direct exposure probe (DEP) and analyzing by GC/MS/methane CI. Prominent ions observed at m/z 183 (benzilic acid), 128 (3-quinuclidinol), 146 and 130 (2-diisopropylaminoethanol), and 97 (methylphosphonic acid) provided additional evidence that the assignments were correct.

2.7 QA/QC Procedures

A detailed description of the QA/QC procedures is outlined in the attached document "Laboratory Quality Control Plan for International Treaty Verification Round Robin Exercises", Appendix H. The use of blanks to eliminate false positives, procedures for instrument calibration, the use of test mixes to monitor instrument performance and other procedures to assure quality control are described for each method of analysis.

Separate extractions using non-deuterated and deuterated solvents, in effect, result in the preparation of two parallel samples, one of the recommended procedures for QA/QC in sample preparation. The NMR samples were prepared first and stored refrigerated in the NMR laboratory separate from the other samples to prevent cross-contamination between the two sets of samples. Having two independent sets of samples provides a check if a particular sample in one set is suspected of contamination.

2.8 Quantitation Methods

GC and GC/MS quantitation were obtained using dibenzothiophene (DBT), one of the instrument performance standards, as an internal standard. DBT was chosen because it gives a very intense molecular ion in both EI (m/z 184) and CI (m/z 185), which makes it an excellent candidate for selected ion monitoring (SIM). A standard mix containing the four compounds identified by NMR and DEP/MS was prepared, derivatized and analyzed prior to addition of the internal standard to verify that DBT did not coelute with the analytes of interest. The internal standard was added after sample preparation and before analysis. Each extract was concentrated to 2 mL (or reconstituted to 2 mL in the case of dried extracts) and 50 μg of DBT added. Samples requiring derivatization were then divided in two, 1 mL derivatized with BSTFA and 1 mL derivatized with diazomethane. Relative response factors were obtained by adding 50 μg of DBT to a cocktail containing 50 μg each of methylphosphonic acid, 2-diisopropylaminoethanol, 3-quinucidinol, and benzilic acid in methanol and derivatizing with both BSTFA and diazomethane.

SIM quantitation was obtained using methane CI (method GC3/MS3). Two ions were used for each compound when possible. The scan time for each ion was 0.07 seconds. Total scan time was 0.7 seconds. Ions (m/z) scanned for methylated samples were 125 (methylphosphonic acid); 146, 130 (2-diisopropylaminoethanol); 185 (DBT); and 225, 227 (benzilic acid). Ions (m/z) scanned for BSTFA derivatized samples were 241, 225 (methylphosphonic acid); 218, 202 (2-diisopropylaminoethanol); 200, 110 (3-quinuclidinol); 185 (DBT); and 283, 91 (benzilic acid).

Both derivatization methods were required to obtain optimum results. 3-Quinuclidinol was best quantitated as the BSTFA derivative. Methylphosphonic acid and benzilic acid were most reliably quantitated using the methyl derivative. Although 2-diisopropylaminoethanol readily derivatized using BSTFA, the most consistent results were obtained by analysis of the underivatized compound in the methylated extracts.

Quantitation was also obtained on selected samples using the same internal standard by GC/FID and full scan GC/MS/EI and CI.

NMR quantitation was obtained by comparison of absolute intensity integrals of the ^{1}H spectra obtained for the water samples against those obtained under identical conditions for an external reference solution containing each of the four identified compounds in D_2O (500 μg/mL each of methylphosphonic acid, 2-diisopropylaminoethanol and benzilic acid, and 750 μg/mL of 3-quinuclidinol). Soil sample analytes were not quantitated by NMR.

Ion chromatography quantitation of benzilic acid and methylphosphonic acid was obtained using the authentic compounds as external standards. Peak areas were compared to those on a linear regression standard curve obtained by serial dilution of a reference standard as described in the HPLC/IC methods section of the QA/QC plan, Appendix H.

Quantitation results are summarized in Appendix I. The highest value obtained by each analytical technique is listed. Unless noted by a footnote, results were obtained using the recommended operating procedures.

3. RESULTS AND DISCUSSION

3.1 Results Using Recommended Operating Procedures

Four Schedule 2 compounds, methylphosphonic acid (MPA), 2-diisopropylaminoethanol (DIAE), 3-quinuclidinol (3-Q) and benzilic acid (BA) were detected in the samples using the recommended operating procedures. Techniques used to identify each compound are summarized in Table 1.

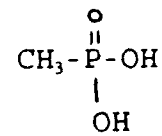
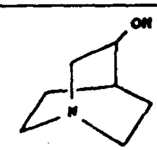
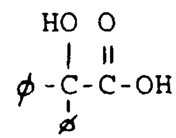
Table 1. Techniques Used To Identify Compounds in Round Robin 4

Compound	Technique				
	GC FID/FPD	GC/MS EI	GC/MS CH_4 CI	NMR	HPLC (IC)
Methylphosphonic Acid	x	x	x	x	
2-Diisopropylaminoethanol	x	x	x	x	x
3-Quinuclidinol	x	x	x	x	
Benzilic Acid	x	x	x	x	x

The structures, formulas and CAS numbers of the four identified compounds are listed in Table 2. A summary of the analytical methods used to identify each compound in each sample is given in Table 3 and Appendix J. Spectroscopic comparison (NMR, full scan GC/MS) to authentic standards is required for unambiguous identification. Chromatographic results (GC, LC and GC/MS/SIM) are reported as tentative unless confirmed by NMR or full scan GC/MS. A summary of the application of each method of analysis follows.

Table 2. Names and Structures of all Reported Compounds

Laboratory: U.S.A. (ERDEC)

Compound Number*	Compound Name and Chemical Abstract Number	Compound Structure	Molecular Formula	Schedule Number
1	Methylphosphonic Acid 993-13-5		CH_5O_3P	2
2	2-Diisopropylaminoethanol 96-80-0	$[(CH_3)_2CH]_2NCH_2CH_2OH$	$C_8H_{19}ON$	2
3	3-Quinuclidinol 1619-34-7		$C_7H_{13}ON$	2
4	Benzilic Acid 76-93-7		$C_{14}H_{12}O_3$	2

* Compound Number Used For Identification in Chromatograms and Spectra

Table 3. Scheduled Compounds Identified In Each Round Robin 4 Sample

Sample Code	Detected Compound	Technique					
		GC/FID	Full Scan GC/MS EI	Full Scan GC/MS CI	GC/MS/CI SIM	HPLC (IC)	NMR
W-15	MPA		x		x	x	x
	DIAE	x	x	x	x		x
	3-Q	x	x	x	x		x
	BA	x	x	x	x	x	x
KEW-14	MPA	x			x	x	x
	DIAE	x	x	x	x		x
	3-Q	x	x	x	x		x
	BA	x	x	x	x	x	x
K-15	MPA						
	DIAE			x	x		
	3-Q				x		
	BA	x		x	x		
SA-15	MPA				x	x	x
	DIAE		x		x		
	3-Q				x		
	BA		x		x	x	
SB-15	MPA				x	x	x
	DIAE				x		x
	3-Q		x	x	x		x
	BA	x	x	x	x	x	x
SN-15	MPA		x	x	x	x	x
	DIAE		x	x	x		
	3-Q		x	x	x		
	BA	x	x	x	x	x	x

3.1.1 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR was the most universal and definitive method of detection employed for the observation of the compounds at the concentration levels used in this round robin, particularly for the water samples. The method is non-destructive and the extracts or concentrated water samples can be analyzed directly with the analytes in the original state rather than being converted to a derivative amenable to GC analysis. The typical GC problems, sample degradation by heat, memory effects (syringe, injection port, and column), and overall column performance are not an issue for NMR analysis.

All four compounds were detected and confirmed unambiguously by comparison to authentic standards in water samples W-15 and KEW-14 and in the soil sample SB-15. The presence of methylphosphonic acid and benzilic acid were confirmed in sample SN-15 and the presence of methylphosphonic acid was confirmed in sample SA-15. Evidence for the other compounds was observed in SN-15 and SA-15. However, because of background interference from these soils, the spectra were not of sufficient quality for this laboratory to report unambiguous identification.

NMR quantitative results obtained for the two water samples W-15 and KEW-14 are summarized in Table 4. Comparison to the original spiking levels of 500 μg for MPA, DIAE and BA and 1000 μg for 3-Q shows that NMR is an excellent technique for quantitation of these compounds in water samples. Comparison of the values for W-15 and KEW-14 provides a measure of how much of each analyte was retained on the C-18 extraction cartridge.

Table 4. NMR Results for W-15 and KEW-14 (μg/50g sample)

Compound	W-15	KEW-14
Methylphosphonic Acid	450	450
2-Diisopropylaminoethanol	400	50
3-Quinuclidinol	900	650
Benzilic Acid	400	350

A compendium of NMR methods, results and spectra are included in Appendix D.

3.1.2 Gas Chromatography (GC)

Gas chromatography using FID and FPD detectors was used in this round robin. Samples were analyzed by FPD in both the phosphorus and sulfur mode. No sulfur-containing scheduled compounds were detected. FPD played a lesser role in this round robin than previously because only one phosphorus-containing compound was detected, methylphosphonic acid. GC methods and selected chromatograms are provided in Appendix F. FID quantitation results for sample extracts obtained using the recommended operating procedures are listed in Table 5 and are included in Appendix J.

Table 5. GC/FID Results Obtained Using ROP

Results in µg/50g Sample				
Water	MPA[1]	DIAE[2]	3-Q[2]	BA[1]
W-15 Neutral Extract B*		89		
Acidic Extract C*				370
Basic Extract D*		126		
Residue E*		51	226	
Total		266	226	370
KEW14 Neutral Extract B*				
Acidic Extract C*				276
Basic Extract D*		60		
Residue E*	83		431	
Total	83	60	431	276
Soil				
SA-15 Water Extract C*				
SB-15 Water Extract C*				220
SN-15 Water Extract C*				200
C-18 Cartridge				
K-15				177

[1] Quantitated as Methyl Derivative
[2] Quantitated as Free Alcohol in Methylated Extract

3.1.3 Gas Chromatography/Mass Spectrometry (GC/MS)

Low resolution mass spectrometry (LRMS) using EI and CI (methane) was used in this exercise. For the purpose of evaluating the ROP, quantitation results obtained for each fraction with adherence to the ROP are provided in Tables 6 and 7 for two different methods of analysis (GC/MS/methane CI SIM and GC/MS/EI ion trap full scan).

Table 6. GC/MS/CI SIM Results Obtained Using ROP

Results in μg/50g Sample				
Water	MPA[1]	DIAE[2]	3-Q[3]	BA[1]
W-15 Neutral Extract B*		107		7
Acidic Extract C*				161
Basic Extract D*		37		3
Residue E*		8	180	
Total		152	180	171
KEW14 Neutral Extract B*		16		
Acidic Extract C*				132
Basic Extract D*		41		
Residue E*		1	130	
Total		58	130	132
Soil				
SA-15 Water Extract C*				
SB-15 Water Extract C*		21	442	248
SN-15 Water Extract C*	65	32	443	216
C-18 Cartridge				
K-15		12	4	48

[1] Quantitated as Methyl Derivative
[2] Quantitated as Free Alcohol in Methylated Extract
[3] Quantitated as TMS Derivative

Table 7. GC/MS ITD Results Obtained Using ROP

Results in μg/50g Sample				
Water	MPA[1]	DIAE[2]	3-Q[3]	BA[1]
W-15 Neutral Extract B*		144		
Acidic Extract C*				549
Basic Extract D*		164		
Residue E*				
Total		308		549
KEW14 Neutral Extract B*		15		
Acidic Extract C*				177
Basic Extract D*		164		
Residue E*				
Total		179		177
Soil				
SA-15 Water Extract C*				
SB-15 Water Extract C*				236
SN-15 Water Extract C*	283	317		155
C-18 Cartridge				
K-15		6		41

[1] Quantitated as Methyl Derivative
[2] Quantitated as Free Alcohol in Methylated Extract
[3] Not Quantitated

The results obtained for the organic extract (A*) and the hexane extracts of the methanol/KOH extracts (D*) for the soils are not listed in the tables because no compounds were detected in any of these extracts by any of the GC/MS methods of analysis. In addition to analyzing these extracts underivatized as recommended in the ROP, extracts A* and D* were derivatized with BSTFA. No scheduled compounds were observed.

Results in Tables 5, 6 and 7 show that the 4 identified compounds were effectively partitioned among the fractions for the water samples. 2-Diisopropylaminoethanol was detected primarily in the organic extracts of the neutral (B*) and basic (D*) water. Benzilic acid was detected primarily in the organic extract of the acidic water (C*). 3-Quinuclidinol was observed exclusively in the remaining residue (E*).

Two problem areas were identified. First, the acidic soil SA-15 was not effectively extracted. No scheduled compounds were detected in any of the SA-15 fractions by GC/MS. Second, methylphosphonic acid, which was previously identified by NMR and IC, was observed in abundance only in sample SN-15. This observation was confirmed using both BSTFA and diazomethane derivatization.

GC/MS operating conditions, chromatograms and mass spectra are included in Appendixes E and F. Quantitation results are included in Appendix J. As shown in Appendix E, EI spectra obtained using the ion trap show similar fragmentation to those obtained using the MSD. However, in some cases, most notably the methyl derivative of MPA and the underivatized DIAE, chemical ionization occurs resulting in protonation of the molecular ion.

3.1.4 Ion Chromatography (IC)

Ion chromatography is a useful method for the detection of ionic species. In this exercise, IC was used for the detection of benzilic acid and methylphosphonic acid in the water samples and in the water extracts of the soil samples. Both were identified and quantitated in all spiked samples. Quantitation results are shown in Table 8. Methods and chromatograms for spiked samples and blanks are included in Appendix G. HPLC/IC was also used to screen for ethyl methylphosphonic acid, isopropyl methylphosphonic acid, pinacolyl methylphosphonic acid, thiodiglycol, thiodiglycol sulfoxide and thiodiglycol sulfone. None of these compounds were detected.

Table 8. Summary of Ion Chromatography Results

Sample Code	Methylphosphonic Acid (μg/sample)	Benzilic Acid (μg/sample)
W-15	500	250
KEW-14	500	150
SA-15	400	100
SB-15	300	200
SN-15	200	200

3.2 Additional Studies

3.2.1 Other Methods of Analysis

To screen for the possible presence of arsenic-containing scheduled compounds, both soil and water samples were analyzed for arsenic using a Perkin-Elmer Atomic Absorption Spectrophotometer (Perkin-Elmer Corporation, Norwalk, CT) equipped with a Zeeman graphite furnace module with nickel nitrate as a modifier., No arsenic was detected in the water sample W-15. Trace levels were observed in the spiked soil SB-15 (1.55 μg/g) and in the soil blank SBB-15 (1.25 μg/g). This difference was too small to report any detectable difference between the sample and blank.

The blank water sample and an in-house distilled water sample were screened for over 40 metals using a Perkin-Elmer Plasma II Inductively Coupled Plasma (ICP) Emission Spectrophotometer (Perkin-Elmer Corporation, Norwalk, CT). The purpose of this analysis was to see if any specific metal cations present in the water sample might be hindering the derivatization of methylphosphonic acid. Detectable metals observed in water blank WB-15 relative to the distilled water sample are listed in Table 9. Of significant interest is the detection of the calcium ion which is present in the water samples at a higher concentration than MPA. It is likely that the formation of the calcium salt hinders derivatization. Removal of calcium ions by passing aqueous samples through a cation exchange resin may solve this problem.

Table 9. Detectable Metals Observed by ICP in Water Blank WB-15

Metal Detected	Concentration (μg/mL)
S	18
Fe	6
Si	30
Ca	28
Ti	0.2
Al	7
Ba	0.07
Na	47
Sr	0.5

3.2.2 Extraction of Soil Sample SA-15

SA-15 was the most acidic of the soils. Reasoning that this may be the cause for the poor extraction efficiency into water, we employed a two-fold approach, 1) pH adjustment of the extraction mix, and 2) a longer extraction time. 7 mL of water and 1 mL methanol were added to 1.5 g of soil. After shaking for 10 minutes the pH was adjusted from 5 to 7 using dilute KOH and the mixture sonicated for 40 minutes. The pH was again readjusted from 5 to 7 and the mixture sonicated for an additional 10 minutes. After allowing the sample to sit overnight, the mixture was filtered, rotovapped to dryness and derivatized with BSTFA in THF. As shown in the summary of quantitative results in appendix I, all 4 identified compounds were observed, but at much lower levels than in the other soil samples. Further investigation is required to effectively extract this type of soil.

3.2.3 Blowdown and BSTFA Derivatization of Water Samples

The most logical approach to the analysis of the water samples is simply to evaporate the water to dryness and derivatize, eliminating the extraction scheme. This approach can be used in this exercise because the most volatile compound observed was 2-diisopropylaminoethanol (bp 190 °C). A 1 mL sample of W-15 was evaporated to dryness and derivatized with BSTFA in THF. The procedure worked well for three of the compounds, benzilic acid, 3-quinuclidinol, and 2-diisopropylaminoethanol. All three were detected in near quantitative yields. No methylphosphonic acid was observed. This was particularly noteworthy, because a standard solution prepared by dissolving the 4 analytes in water followed by blowdown and BSTFA derivatization produced excellent derivatization of all four compounds, including methylphosphonic acid.

3.2.4 Methylphosphonic Acid Derivatization

The most puzzling aspect of this exercise was the difficulty experienced in the detection of MPA by GC/MS. The derivatization method worked nicely for the reference standard and the soil sample SN-15, using both diazomethane (Diazald as the presursor) and BSTFA/THF. Since the water extracts of the soil were not pH adjusted, and MPA was observed in the neutral soil SN-15, but not the basic soil SB-15, it was rationalized that perhaps MPA was not being derivatized because it was tied up in the salt (basic) form. Further evidence that the samples were basic was provided by the observation that addition of the diazomethane resulted in the persistence of the yellow color, suggesting no excess acid was present. To test this theory, both methylated and BSTFA derivatized samples were treated with acidic methanol in an attempt to improve derivatized MPA yield. Methylated samples were blown down almost to dryness and dissolved in 1 mL of acidic methanol and rederivatized with diazomethane. The most notable improvement was with the neutral soil SN-15. The quantity of MPA detected by GC/MS/CI SIM was increased from 65 to 239 μg/sample. The quantity detected in SB-15 increased from 0 to 10 μg/sample. No MPA was detected in the water samples. The continued persistence of diazomethane color suggests that perhaps the acidic methanol was not sufficient to neutralize the salts. No further diazomethane derivatization was performed.

The 1 mL BSTFA-derivatized samples were also treated with acidic methanol by evaporating the derivatized sample to dryness, dissolving in 1 mL of acidic methanol, evaporating to dryness again and rederivatizing with BSTFA/THF at 60 °C for an additional 20 minutes. The first treatment of sample W-15 resulted in the detection of a trace amount of MPA. A second similar treatment resulted in the detection of 174 μg/sample. A comparison of the chromatograms obtained before and after acidification is shown in Figure 1. A single treatment of sample KEW-14 was also done and resulted in the detection of 10 μg/sample. A second treatment was not performed.

Although additional studies are required to confirm these results, it appears that control of sample acidity or removal of the interfering cations, particularly calcium as noted above, with a cation exchange resin may be crucial for effective derivatization of methylphosphonic acid in soil and water matrices.

3.3 Evaluation of C-18 Extraction Cartridge

The most indicative results that evaluate the retention efficiency of the C-18 cartridge for each compound are the NMR data in Table 4. The results indicate 87% of the 2-diisopropylaminoethanol, 28% of the 3-quinuclidinol, 12% of the benzilic acid and none of the methylphosphonic acid were retained on the cartridge. The GC/MS data qualititatively agrees with this assessment with 62%, 28% and 23% retention being observed, respectively, for the first three compounds, based on SIM values for W-15 and KEW-14 using the ROP data (Table 6). All compounds except methylphosphonic acid were observed by GC/MS/CI/SIM in the sample K-15. However, the sum of the levels detected in K-15 and KEW-14 were lower than the levels observed in W-15. This may be traced to the selection of acetonitrile as the extraction solvent. Perhaps acetone or some other solvent would be more suitable. For the four compounds detected in this study, shipping the unextracted water was clearly preferrable to using

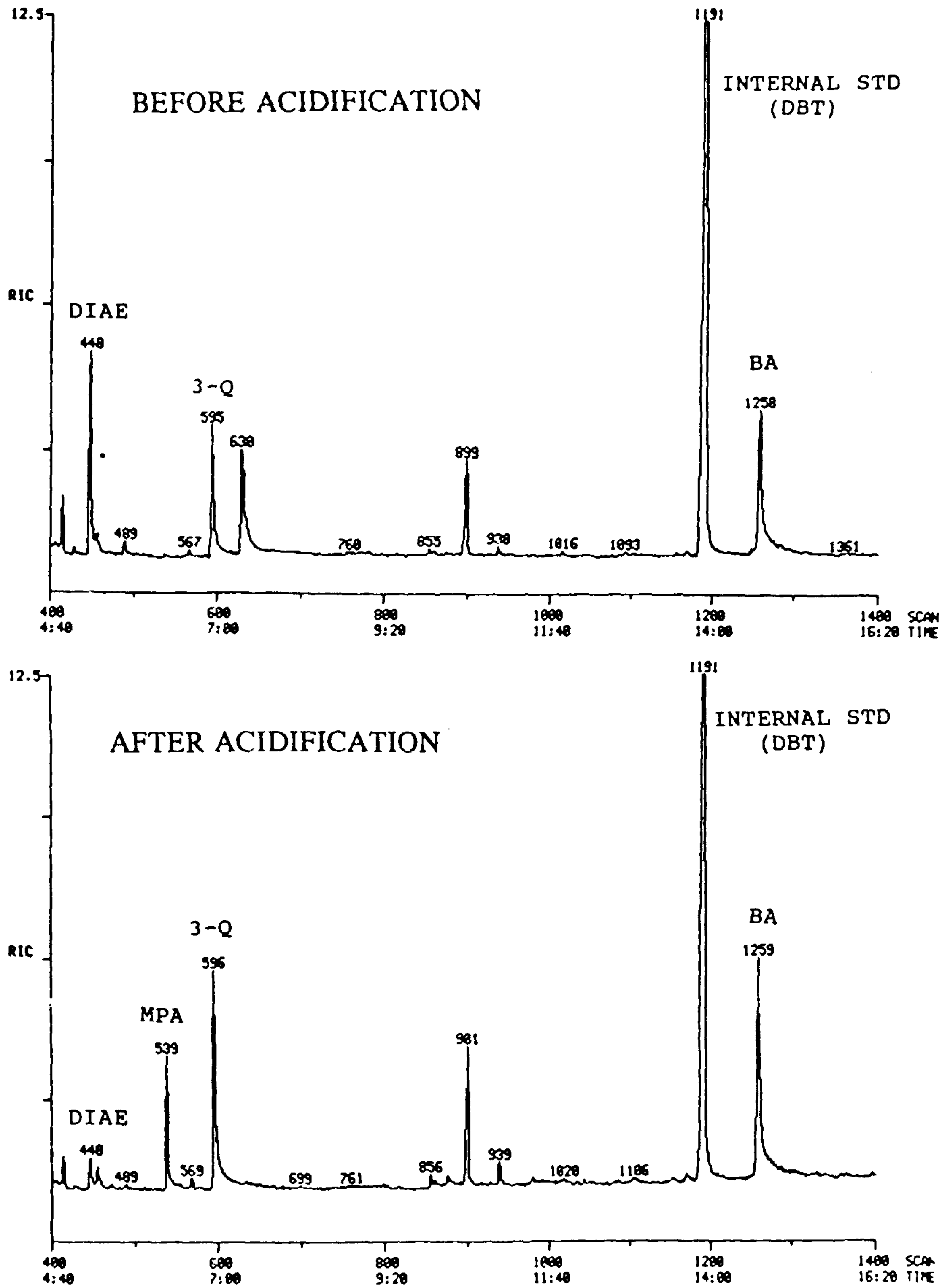


Figure 1. GC/MS/CI/SIM Chromatograms of TMS Derivative of Sample W-15 (1 mL) Before and After Acidification

the C-18 cartridge, resulting in higher quantities detected. No degradation of the analytes was observed in the water samples. Use of a C-18 cartridge may be more applicable for preservation when actual agents are involved which may degrade in water during shipping and storage. It should be noted that the validity of the results from this laboratory may be questionable because the cartridge (K-15) we received did not correspond to the water sample received (KEW-14).

4. CONCLUSIONS

Four Schedule 2 compounds (methylphosphonic acid, 2-diisopropylaminoethanol, 3-quinuclidinol and benzilic acid) were correctly identified and quantitated in the Round Robin 4 soil and water samples. The compounds were identified unambiguously by comparison to authentic reference standards by NMR, GC/FID/FPD, GC/MS (EI and CI) and ion chromatography.

NMR and ion chromatography were the preferred methods of analysis of the acids and amino-alcohols encountered in this exercise. Direct analysis of water samples using these techniques produced outstanding quantitation results. Sample extraction and derivatization required for gas chromatographic analysis resulted in lower quantitation levels and greater variability.

All four compounds were detected in the aqueous extract of the soil samples. The analytes were effectively partitioned among the extracts of the aqueous samples. DIAE was detected primarily in the neutral and basic extracts, BA primarily in the acidic extract, and 3-Q exclusively in the residue remaining after extraction.

The acidic humus-type soil SA-15 proved difficult to extract. Low yields indicate the analytes are tightly bound to the soil and not efficiently extracted using the procedures applied here. Further work is required with this type of soil to increase the extraction efficiency.

Two methods of derivatization (trimethylsilylation with BSTFA and methylation with diazomethane) were used to obtain optimum results. MPA and BA were best quantitated as the methyl derivative, 3-Q as the TMS derivative, and DIAE as the underivatized alcohol.

Problems were encountered with all samples except soil sample SN-15 with respect to both BSTFA and diazomethane derivatization of MPA using the ROP. Acidification of selected samples resulted in improved yields. ICP analysis identified calcium as the most likely ion causing salt formation and inhibition of derivatization. Acidification or the use of cation exchange resins to remove interfering ions is required for effective MPA derivatization.

For the four compounds detected in this study, shipping the unextracted water was preferrable to using a C-18 solid phase extraction cartridge. No degradation of the analytes in the water was observed. Results indicate 87% of the DIAE, 28% of the 3-Q, 12% of the BA and none of the MPA was retained on the cartridge. The effectiveness of the C-18 cartridge was further reduced because elution yields of the analytes from the cartridge were low with acetonitrile.

The problems encountered with MPA derivatization and the ease with which the direct methods NMR and HPLC were able to readily detect MPA emphasizes the importance of using complementary techniques for the analysis of treaty verification samples.

APPENDIX A

CORRESPONDENCE PROVIDED ON ROUND ROBIN 4 SAMPLE PREPARATION

PREPARATION OF SAMPLES FOR ROUND ROBIN 4

RAYMOND MCGUIRE
RODNEY EAGLE
JEFFREY HAAS
RICHARD WHIPPLE

LAWRENCE LIVERMORE NATIONAL LABORATORY

The samples for Round Robin 4 were produced with the idea of promoting the comparison of techniques for preparing environmental samples (soil and water) for instrumental analysis. In other words, a comparison of the various extraction techniques used in the participating laboratories. Thus each of the samples contained the same four spiking compounds: methylphosphonic acid (MPA), diisopropylaminoethanol (DIAE), 3-quinuclidinol (3Q), and benzilic acid (BA).

These materials were chosen as being likely products of decomposition of VX and BZ. They also are moderately water soluble and, because of their polarity, will adhere to soils.

Three types of soils were chosen: a relatively neutral sandy loam with a high silica content, a slightly basic desert alkali soil with a high carbonate content, and a slightly acidic forest humus with a moderately high organic content. A water solution of the spiking materials was mixed into the soil as homogeneously as possible to give a concentration of 0.01 mg/gram of soil (10 ppm), with the exception of the 3Q where a concentration of 0.04 mg/gram of soil (40 ppm) was used.

The water samples were made from relatively clean water from an aqueduct which was filtered through a one micron nucleopore filter prior to spiking. The water was slightly basic at a pH of 8.2. The concentrations of the spiking materials in the water were the same (0.01 mg/ml or 10 ppm) as on the soil, again with the exception of the 3Q which had a concentration of 0.02 mg/ml or 20 ppm in the water. Water samples were divided into two 50 ml aliquots. One of these was passed through the C-18 cartridge (both the cartridge and the efluent water were sent). Methylene chloride was added to the other water aliquot at a ratio of 0.5 ml of CH_2Cl_2 per liter of water.

All of the samples were packaged in cleaned glass bottles for shipment.

Blank

APPENDIX B

CHAIN OF CUSTODY PLAN

IOP No. 001.00
1 Mar 93
Revision No 0.0

Internal Operating Procedure

CHAIN OF CUSTODY, LABORATORY SAMPLE DISTRIBUTION, AND SAMPLE DOCUMENTATION FOR ROUND ROBIN TEST #4

1.0 SCOPE AND PURPOSE:

1.1 The U.S. Army Edgewood Research, Development and Engineering Center (ERDEC) is participating in Round Robin Test #4 for the verification of chemical disarmament.

1.2 This Internal Operating Procedure (IOP) covers the *chain-of-custody* requirements needed to ensure safe handling and proper documentation for the Round Robin Test #4.

2.0 REFERENCES:

2.1 International Inter-laboratory Laboratory Comparison (Round Robin) Test for the Verification of Chemical Disarmament. Helsinki 1992. ISBN 951-6272-X.

2.2 Laboratory Quality Control Plan for Chemical Agent Standard Analytical Reference Material. R&TD, USAERDEC, 15 Nov 1992.

2.3 Laboratory Quality Control Plan for International Treaty Verification Round Robin Exercises, R&TD, draft Feb 92..

2.4 EAI Corporation Standing Operating Procedure No. EA-1. ERDEC Laboratory Ropoms 172, 176, 178, and 181 of Building E-3330, Research Directorate.

3.0 SAFETY AND SURETY: Safety and surety as stipulated in CRDEC GOP# 8-0-90-0000 ("General Operating Procedures for Surety Work") and CRDEC SOP #8-0-90-0007 ("General Provisions for Exempt Chemical Surety Material (XCSM)"), CRDEC SOP #8-0-90-0011 ("Analysis of Samples from Building Demolition, Ton Containers, and Questionable Origin, Plus Routine Analytical Samples") will be adhered to when using this IOP.

4.0 SUMMARY OF PROCEDURE: Samples for the Round Robin Test #4 are non-hazardous. The samples are of three different types of soil plus a water sample and C-18 cartridge.

Each sample will have a chain-of-custody record to ensure the authenticity of the evidence collected. Each sample will be distributed within the laboratory in accordance with section 7.6 of this IOP. All documents associated with the sample will be kept on file or at appropriate storage by the Analytical Methodology Team for at least seven years.

5.0 MATERIALS AND EQUIPMENT: N/A

6.0 CALIBRATION (STANDARDS PREPARATION): N/A

7.0 PROCEDURE:

7.1 INTRODUCTION. The Analytical Methodology Team will be responsible for receiving the samples into the laboratory, performing analysis on the samples, and maintaining records associated with each sample.

7.1.1 For the Chain-of-Custody process, the DA Form 4137 will be used to create an audit trail for each sample (see attachment 1). The DA Form 4137 lists all transfers in the possession of the samples. This piece of documentary evidence attests that the sample was constantly under custody during laboratory analysis. A sample is under custody if: (l) it is under possession, or (2) it is in a person's view, after being in possession or (3) it is under possession and locked up, or (4) it is in a designated secure area.

7.1.2 All documents associated with the sample (e.g. DA Form 4137, laboratory notebooks, sample iog-in sheets, sample control records, correspondence, QC charts, photos, etc.) will be maintained on file by the Analytical Methodology Team for at least seven years.

7.2 CHAIN-OF-CUSTODY FORM. The Chain-of-Custody record, DA Forms 4137, will be crossed checked with the labels on the samples received. The forms will be numbered consecutively and located in the "Serial Number" section of the form. The number will reflect: The type of sample (S=soil, W=water, and C=cartridge). The blank soil samples are marked with the word "blank" on the label. The blank soil samples are labeled to correspond to the the spiked samples; i.e. SAB is the blank for SA, SBB is the blank for sample SB, and SNB is the blank for SN. The last character in the number is the laboratory number. The laboratory number for the R&TD laboratory is number 15.

7.2.1 The receiving activity is the R&TD laboratory in Building E-3300. The sample custodian will be Michael Lochner as the primary with Dennis Rohrbaugh as the alternates. Other alternates will be appointed as required.

7.3 SAMPLE LABELS. If the documentation and labels agree, the DA Form 4137 will be signed by the sample custodian.

7.4 SAMPLE DOCUMENTATION. The sample custodian will complete the Sample Log-In Sheet, Chemistry Department.

7.4.1 The sample custodian will record the date, page number, custodian, site ID, and transportation shipping number of the sample.

7.4.2 The sample custodian will indicate the state of the custody seal, the presence/absence of the chain-of-custody records, integrity of bottle contents, pertinent sample information, and sample tag (option) information on the Sample Log-In Sheet, Chemistry Department.

7.4.3 The sample custodian will record the date and time the sample was received, the chain-of-custody record number, site identification, sample tag number (option), assigned lab number, and remarks.

7.4.4 The sample custodian will record the new sample in the general sample log. Comments, as described in 7.4.1-7.4.3, about the individual sample(s) will be made. Observations about the sample, such as, color, consistency and viscosity will be made on the data sheet.

7.5 STORAGE OF EXTRACTS/SAMPLES

7.5.1 The extracted/prepared samples will be placed in a sample tray.

7.5.2 The sample tray will be placed in a container and secured in a locked refrigerator. The analysts responsible for the appropriate procedure will be contacted by the sample custodian that the samples are ready for analysis. The DA Form 4137 will be placed in the tray with the samples and will follow the samples as they are routed from lab to lab.

7.6 DISTRIBUTION OF EXTRACTS/SAMPLES. Distribution of extracted/prepared samples and standard reference solutions for analysis will be performed by the sample custodian.

7.6.1 Sample vials will be placed in a sample tray. Standard reference solution vials will be distributed first. Then sample vials (in the following order: GC-FID, GC-FPD, GC/MS, ICD & NMR analysis) will be distributed.

7.6.2 The sample custodian will record the sample number, description, date and time the sample(s) were given to the analyst, the reason for distributing the sample (i.e., type of analysis Performed, the room and building the samples are being taken to, and the analyst's name and initials) on the Sample Control Record, Chemistry Department. In addition, the receiving analyst will sign DA Form 4137 as "received by". The sample custodian will sign DA Form 4137 as "released by".

7.6.3 After analyses are completed, the analyst will notify the sample custodian to pick up the samples. The samples will be processed by the sample custodian by recording the date and time the sample was received, with the custodian's signature. In addition, the sample custodian will sign DA Form 4137 as "received by". The analyst will sign DA Form 4137 as "released by".

7.7 DISPOSAL OF SAMPLES AND STANDARDS. The sample custodian will handle & dispose of samples & standards in accordance with GOP # 8-0-90-0000, SOP # 8-0-9-90-0009, and SOP # 8-0-90-0011.

8.0 CALCULATIONS: N/A.

9.0 QUALITY CONTROL: N/A

EVIDENCE/PROPERTY CUSTODY DOCUMENT For use of this form see AR 190-45 and AR 195-5; the proponent agency is US Army Criminal Investigation Command	MPR CID SEQUENCE NUMBER CRD REPORT/CID ROI NUMBER

RECEIVING ACTIVITY	LOCATION	
NAME, GRADE AND TITLE OF PERSON FROM WHOM RECEIVED ☐ OWNER ☐ OTHER	ADDRESS (Include Zip Code)	
LOCATION FROM WHERE OBTAINED	REASON OBTAINED	TIME/DATE OBTAINED

ITEM NO	QUANTITY	DESCRIPTION OF ARTICLES (Include model, serial number, condition and unusual marks or scratches)

CHAIN OF CUSTODY

ITEM NO	DATE	RELEASED BY	RECEIVED BY	PURPOSE OF CHANGE OF CUSTODY
		SIGNATURE NAME, GRADE OR TITLE	SIGNATURE NAME, GRADE OR TITLE	
		SIGNATURE NAME, GRADE OR TITLE	SIGNATURE NAME, GRADE OR TITLE	
		SIGNATURE NAME, GRADE OR TITLE	SIGNATURE NAME, GRADE OR TITLE	
		SIGNATURE NAME, GRADE OR TITLE	SIGNATURE NAME, GRADE OR TITLE	
		SIGNATURE NAME, GRADE OR TITLE	SIGNATURE NAME, GRADE OR TITLE	

DA FORM 4137, 1 JUL 76 — Replaces DA FORM 4137, 1 Aug 74 and DA FORM 4137-R Privacy Act Statement 26 Sep 75 Which are Obsolete

LOCATION ____________ DOCUMENT NUMBER ____________

APPENDIX C

SAMPLE PREPARATION

Table C-1. NMR Extraction Weights and Volumes

NMR SAMPLES		(A) CDCL3			(B) D2O			(C) 0.5M KOH/CH3OH		
SAMPLE	WEIGHT/ MLS	MLS USD	MLS RCV'D	MLS CNC	MLS USD	MLS RCV'D	MLS CNC	MLS USD	MLS RCV'D	RECONC FM DRY
SAB-15	10.058	20.0	11.8	1.6	20.0	14.0	2.9	20.0	18.2	1.5
SA-15	10.068	20.0	13.0	1.7	20.0	15.7	3.3	20.0	15.1	1.5
SBB-15	10.001	20.0	16.9	1.9	20.0	15.4	2.2	20.0	19.6	1.5
SB-15	10.057	20.0	16.6	2.2	20.0	15.4	3.6	20.0	19.4	1.5
SNB-15	10.053	20.0	17.1	1.6	20.0	17.4	2.0	20.0	17.6	1.5
SN-15	10.023	20.0	15.1	2.3	20.0	14.8	2.6	20.0	18.7	1.5

SAMPLE	(A) MLS/MLS	(B) CDCL3 MLS USD	MLS RCVD	AQUE TO DRY	REDISS IN D2O
WB-15	1.0/9.0	1.5	0.75	8.1	1.0
W-15	1.0/9.0	1.5	0.75	8.1	1.0
KEW-14	1.0/9.0	1.5	0.75	8.3	1.0

Table C-2. GC/MS Extraction Weights and Volumes

GC/MS SAMPLES

SAMPLE	WEIGHT/ MLS	(A*) CH2CL2 MLS USD	MLS RCVD	MLS CNC	MLS USD	(B*) RCVD TO HPLC	HPLC H2O MLS RCVD TO DRY	(C*) CH3OH MLS REDISS	(D*) 0.5M KOH/ CH3OH MLS USD	MLS RCVD
SAB-15*	10.033	20.0	7.1	2.0	20.0	1.0	8.5	2.0	4.0	2.9
SA-15*	10.083	20.0	8.5	2.0	20.0	1.0	10.1	2.0	4.0	2.6
SBB-15*	10.047	20.0	12.5	2.4	20.0	1.0	12.7	2.0	4.0	2.8
SB-15*	10.061	20.0	15.9	2.0	20.0	1.0	13.0	2.0	4.0	2.8
SNB-15*	10.009	20.0	11.5	2.1	20.0	1.0	11.8	2.0	4.0	2.6
SN-15*	10.031	20.0	15.9	1.6	20.0	1.0	9.9	2.0	4.0	2.7

SAMPLE	(A*) WATER MLS MLS HPLC/USD	(B*) CH2CL2 MLS USD	MLS RCVD	MLS CNC	(C*) CH2CL2 ACID PH MLS USD	MLS RCVD	MLS CNC	(D*) CH2CL2 BASIC PH MLS USD	MLS RCVD	MLS CNC	(E*) CH3OH FROM DRY MLS USD
WB-15*	1.0/9.0	5.0	4.0	1.8	5.0	4.2	2.0	5.0	4.3	2.0	2.0
W-15*	1.0/9.0	5.0	3.9	2.0	5.0	4.0	2.0	5.0	3.9	2.0	2.0
KEW-14*	1.0/9.0	5.0	3.8	2.0	5.0	4.1	1.9	5.0	4.1	2.2	2.0

C18 SAMPLE	ACETONITRILE MLS USD
KB-15	1.0
K-15 LOADED	1.0

Table C-3. Preparation of Soil Samples For Chromatographic Analysis

Country: U.S.A. (ERDEC)	Sample Code: SA-15, SAB-15, SB-15, SBB-15, SN-15, SNB-15
Date Samples Received: 16 February 1993	Storage Temperature: +6 °C
Storage Time of Samples: 14 Days	Soil Type: Humus (SA), Sandy (SB), Clay (SN)
EXTRACTION	DERIVATIZATION
Method: Ultrasonic Bath (ice)	Extract Derivatized: H_2O
Solvents/Volume: 1. CH_2Cl_2 2 x 10 mL 2. Deionized HPLC Water 2 x 10 mL 3. 0.5M KOH/CH_3OH 2 x 10 mL	Volume: 1. 1 mL 2. 1 mL Derivatizing Agent: 1. BSTFA/THF 2. Diazomethane Reaction Temperature: 1. 60 °C 2. *Room Temperature*
Extraction Time: 10 min	Reaction Time: 1. 20 min 2. 20 min
Concentration Method: Rotovap for H_2O Extracts, N_2 Flow for Others	Concentration Method: 1. None 2. N_2 Flow
Final Volume: 2 mL	Final Volume: 1. 0.5 mL 2. 1.0 mL

Comments
1. See Scheme C-1.
2. Samples obtained from this reaction scheme are identified with an asterisk (*).
3. Aqueous layer redissolved in methanol prior to derivatization.
4. Initial organic layer filtered through a 0.45 μm filter; chlorinated solvents leach plasticizers from the recommended 0.45 μm disposable cartridge filters.
5. Third extraction with KOH/CH_3OH was then extracted with hexane.
6. See Table C-2 for measured volumes.

Table C-4. Preparation of Soil Samples For NMR Analysis

Country: U.S.A. (ERDEC)	Sample Code: SA-15, SAB-15, SB-15, SBB-15, SN-15, SNB-15
Date Samples Received: 16 February 1993	Storage Temperature: +6 °C
Storage Time of Samples: 14 Days	Soil Type: Humus (SA), Sandy (SB), Clay (SN)
EXTRACTION	DERIVATIZATION
Method: Ultrasonic Bath (ice)	Extract Derivatized: Selected D_2O Extracts
Solvents/Volume: 1. $CDCl_3$ 2 x 10 mL 2. D_2O 2 x 10 mL 3. 0.5M KOH/CH_3OH 2 x 10 mL	Volume: 1 mL Derivatizing Agent: 0.25 mL BSTFA, 0.25 mL THF Reaction Temperature: 60 °C
Extraction Time: 1. 10 min 2. 5 min 3. 10 min	Reaction Time: 20 min
Concentration Method: Dry N_2	Concentration Method: None
Final Volume: 1. 1 mL 2. 1 mL 3. Residue dissolved in 1.5 mL CD_3OD	Final Volume: 0.5 mL

Comments

1. See Scheme C-2.
2. Initial organic layer filtered through a 0.45 μm filter; chlorinated solvents leach plasticizers from the recommended 0.45 μm disposable cartridge filters.
3. See Table C-1 for measured volumes.

Table C-5. Preparation of Water Samples For Chromatographic Analysis

Country: U.S.A. (ERDEC)	Sample Code: W-15, WB-15, KEW-14
Date Samples Received: 16 February 1993	Storage Temperature: +6 °C
Storage Time of Samples: 14 Days	
EXTRACTION	DERIVATIZATION
Method: Shaking	Extract Derivatized: All Extracts
Solvents/Volume: 1. CH_2Cl_2 (Neutral) 2 x 2.5 mL 2. CH_2Cl_2 (pH<2) 2 x 2.5 mL 3. CH_2Cl_2 (pH>10) 2 x 2.5 mL 4. Dried Residue Dissolved in CH_3OH	Volume: 1. 1 mL 2. 1 mL Derivatizing Agent: 1. BSTFA/THF 2. Diazomethane Reaction Temperature: 1. 60 °C 2. Room Temp
Extraction Time: 5 min	Reaction Time: 1. 20 min 2. 20 min
Concentration Method: Dry N_2	Concentration Method: 1. None 2. N_2 Flow
Final Volume: 1. 2.0 mL 3. 2.0 mL 2. 2.0 mL 4. 2.0 mL	Final Volume: 1. 0.5 mL 2. 1.0 mL

Comments

1. See Scheme C-3.
2. See Table C-2 for measured volumes.

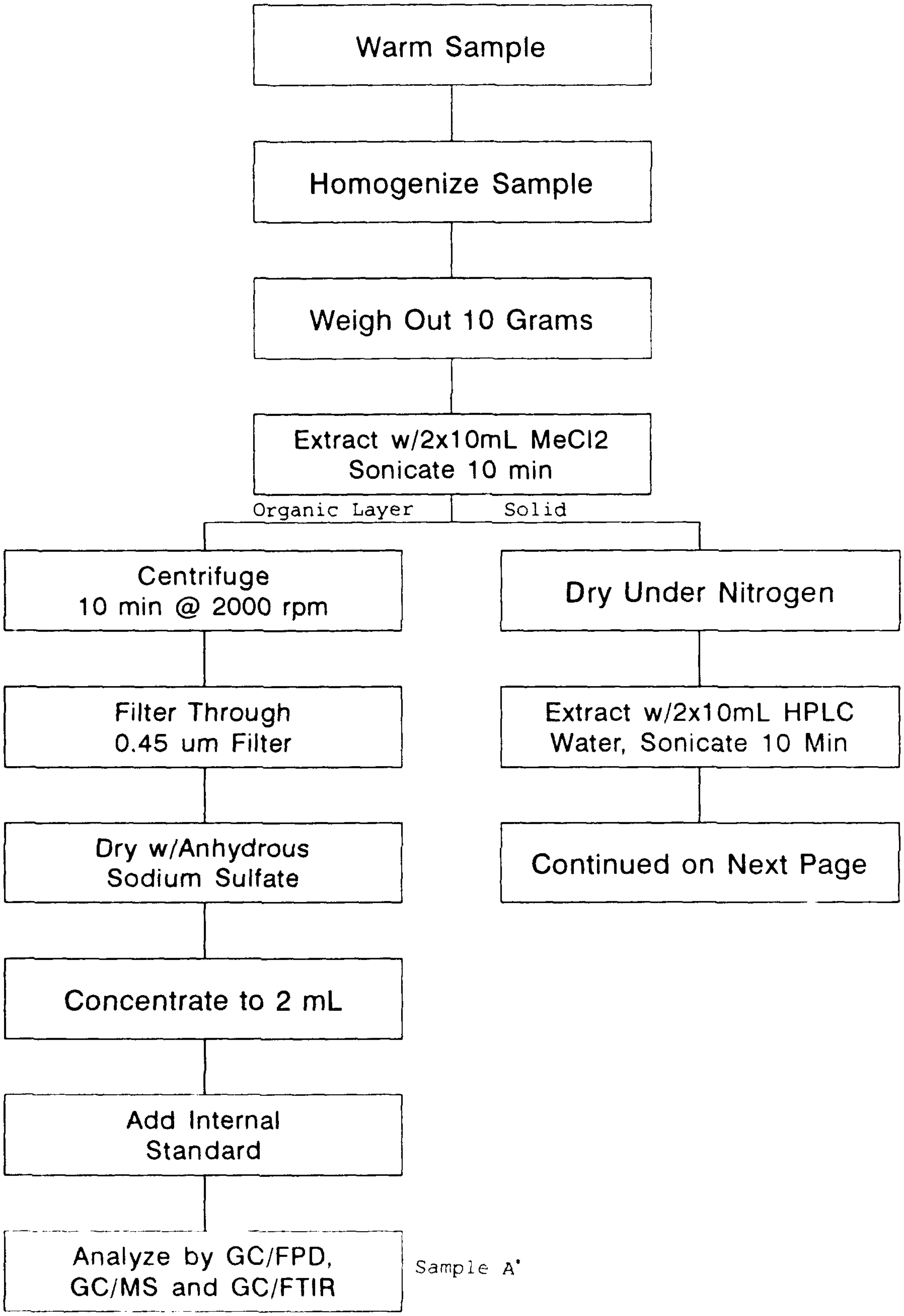
Table C-6.. Preparation of Water Samples For NMR Analysis

Country: U.S.A. (ERDEC)	Sample Code: W-15, WB-15, KEW-14
Date Samples Received: 16 February 1993	Storage Temperature: +6 °C
Storage Time of Samples: 14 Days	
EXTRACTION	DERIVATIZATION
Method: Shaking	Extract Derivatized: Selected D_2O Extracts
Solvents/Volume: 1. $CDCl_3$ 2 x 0.75 mL 2. Water Residue Evaporated to Dryness and Dissolved in 1 mL D_2O	Volume: 1 mL Derivatizing Agent: BSTFA/THF Reaction Temperature: 60 °C
Extraction Time: 5 min	Reaction Time: 20 min
Concentration Method: Dry N_2	Concentration Method: None
Final Volume: 1. 1.5 mL 2. 1.0 mL	Final Volume: 0.5 mL
Comments 1. See Scheme C-4. 2. See Table C-1 for measured volumes.	

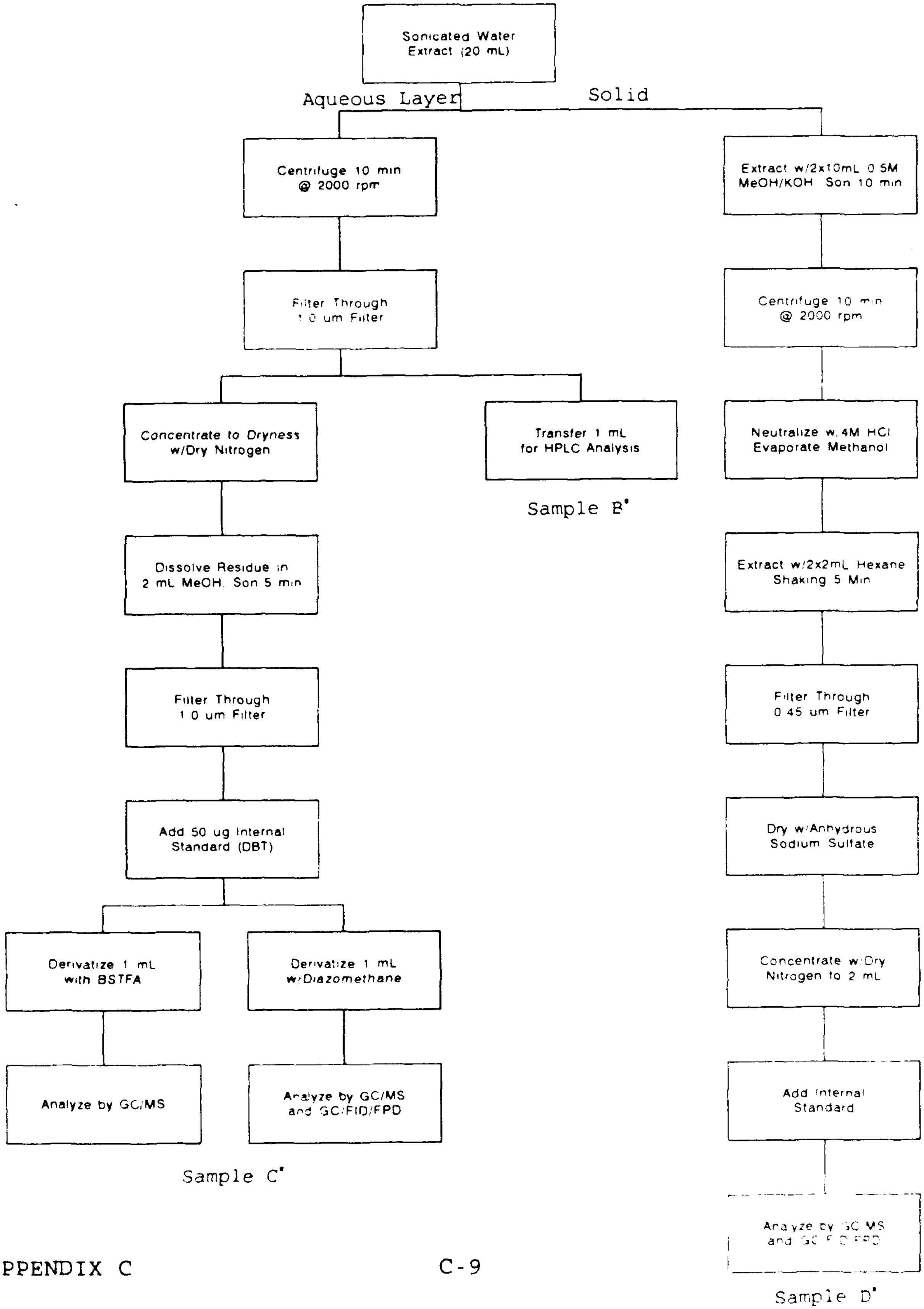
Table C-7. Preparation of Solid Phase Extraction Cartridges

Country: U.S.A. (ERDEC)	Sample Code: K-15, KB-15
Date Samples Received: 16 February 1993	Storage Temperature: +6 °C
Storage Time of Samples: 14 Days	
EXTRACTION	DERIVATIZATION
Method: Elution	Extract Derivatized: All Extracts
Solvents/Volume: Acetonitrile 5 mL	Volume: 1. 1 mL 2. 1 mL
	Derivatizing Agent: 1. BSTFA/THF 2. Diazomethane
	Reaction Temperature: 1. 60 °C 2. Ambient
Extraction Time:	Reaction Time: 1. 20 min 2. 20 min
Concentration Method: None	Concentration Method: 1. None 2. N_2 Flow
Final Volume: 5 mL	Final Volume: 1. 0.5 mL 2. 1.0 mL
Comments See Scheme C-5.	

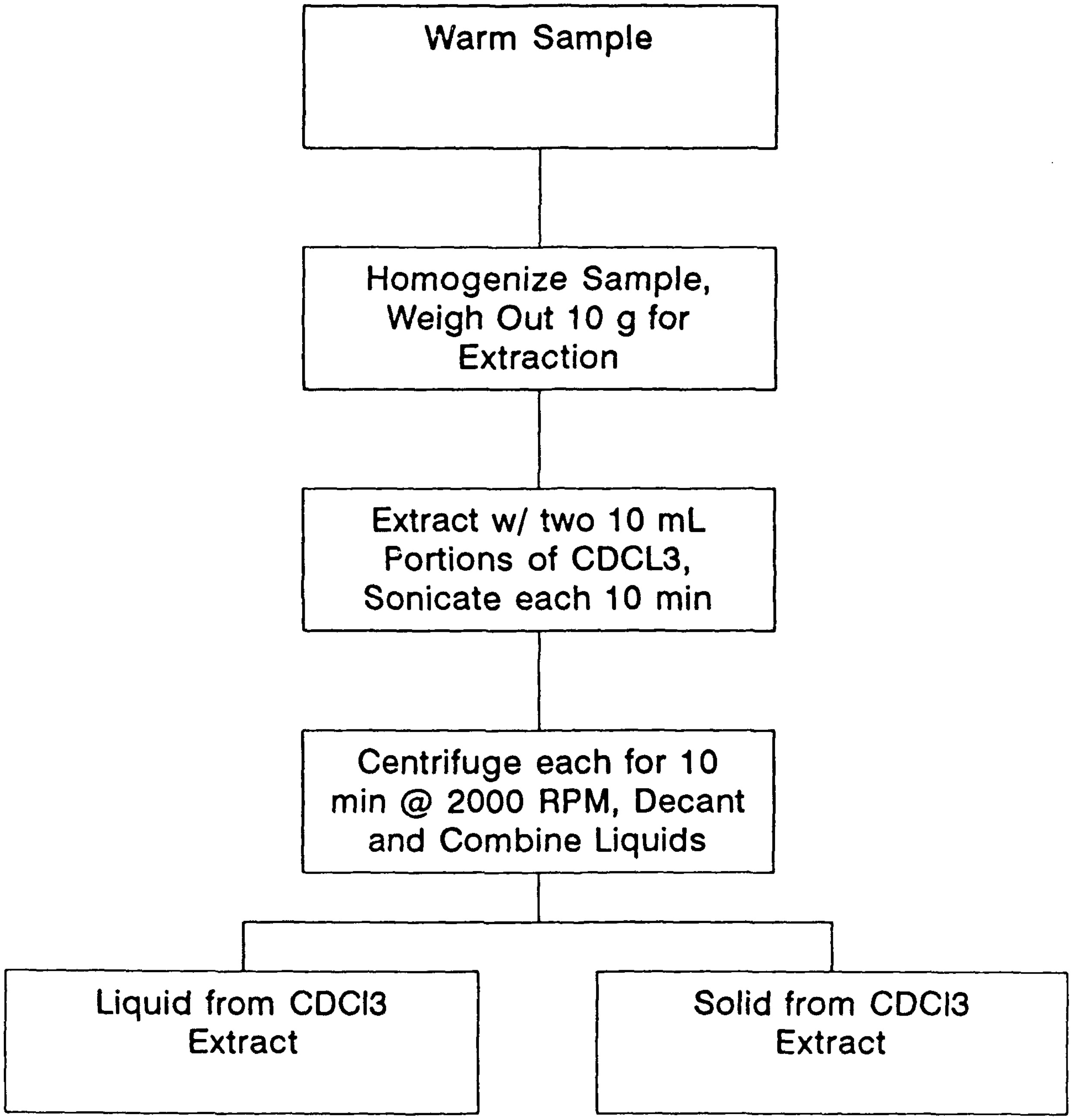
Scheme C-1. Soil Sample Preparation For Chromatographic Analysis



Scheme C-1. Soil Sample Preparation For Chromatographic Analysis (cont'd)



Scheme C-2. Soil Sample Preparation For NMR Analysis



Scheme C-2. Soil Sample Preparation For NMR Analysis (Cont'd)

Liquid from CDCl3 Extract

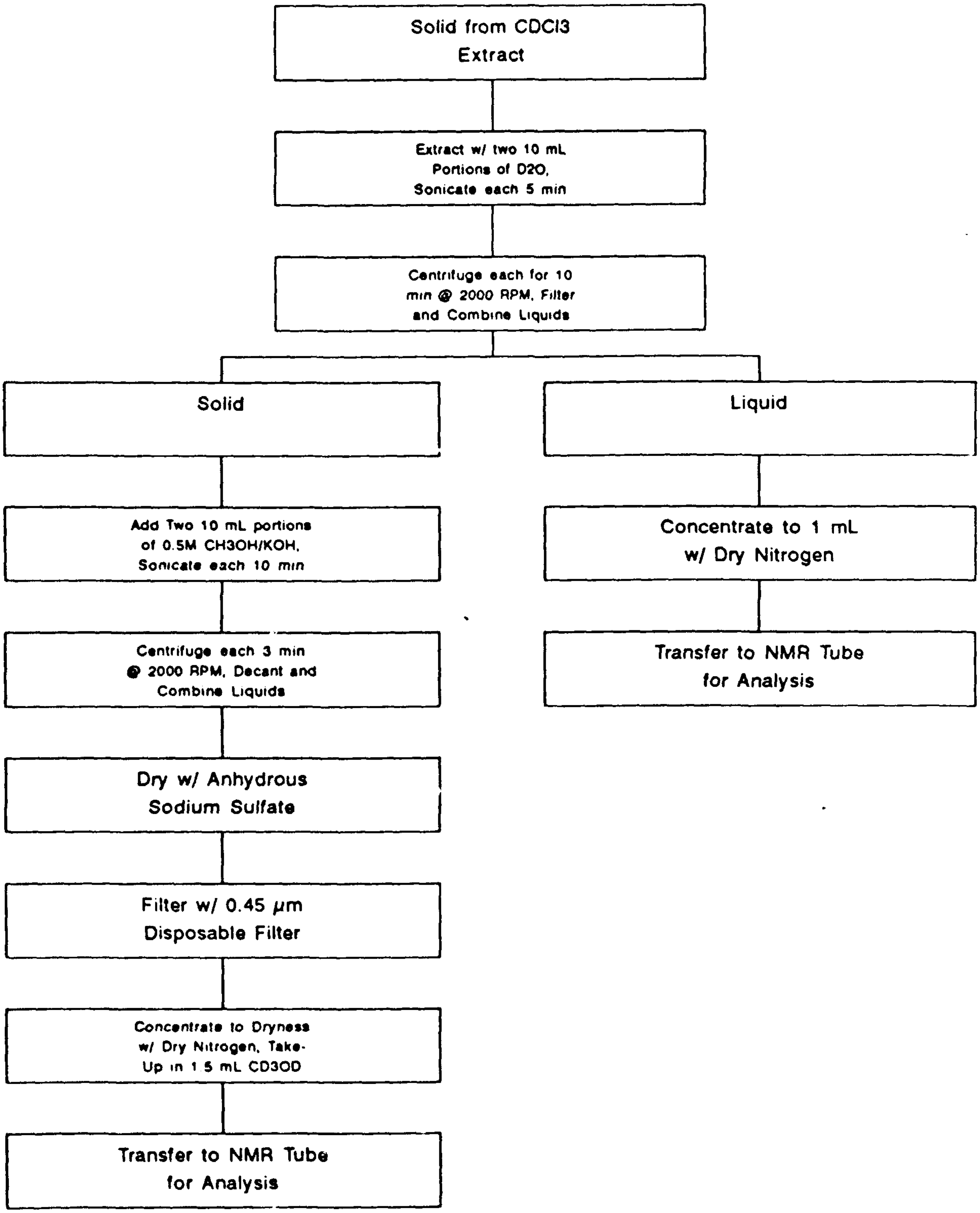
Dry w/ Anhydrous Sodium Sulfate

Filter thru 0.45 μm Disposable Filter

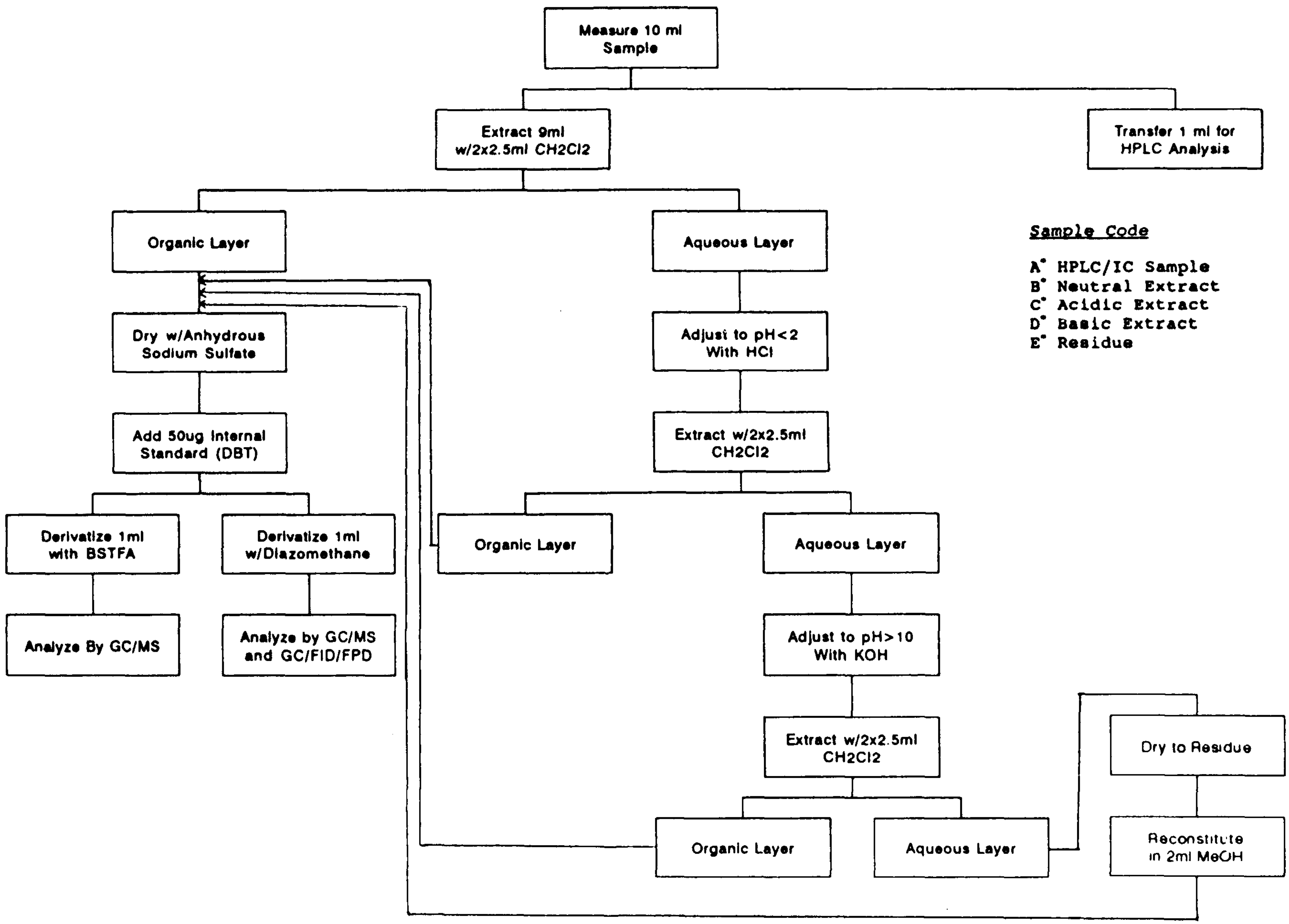
Concentrate to 1 mL w/ Dry Nitrogen

Transfer to NMR Tube for Analysis

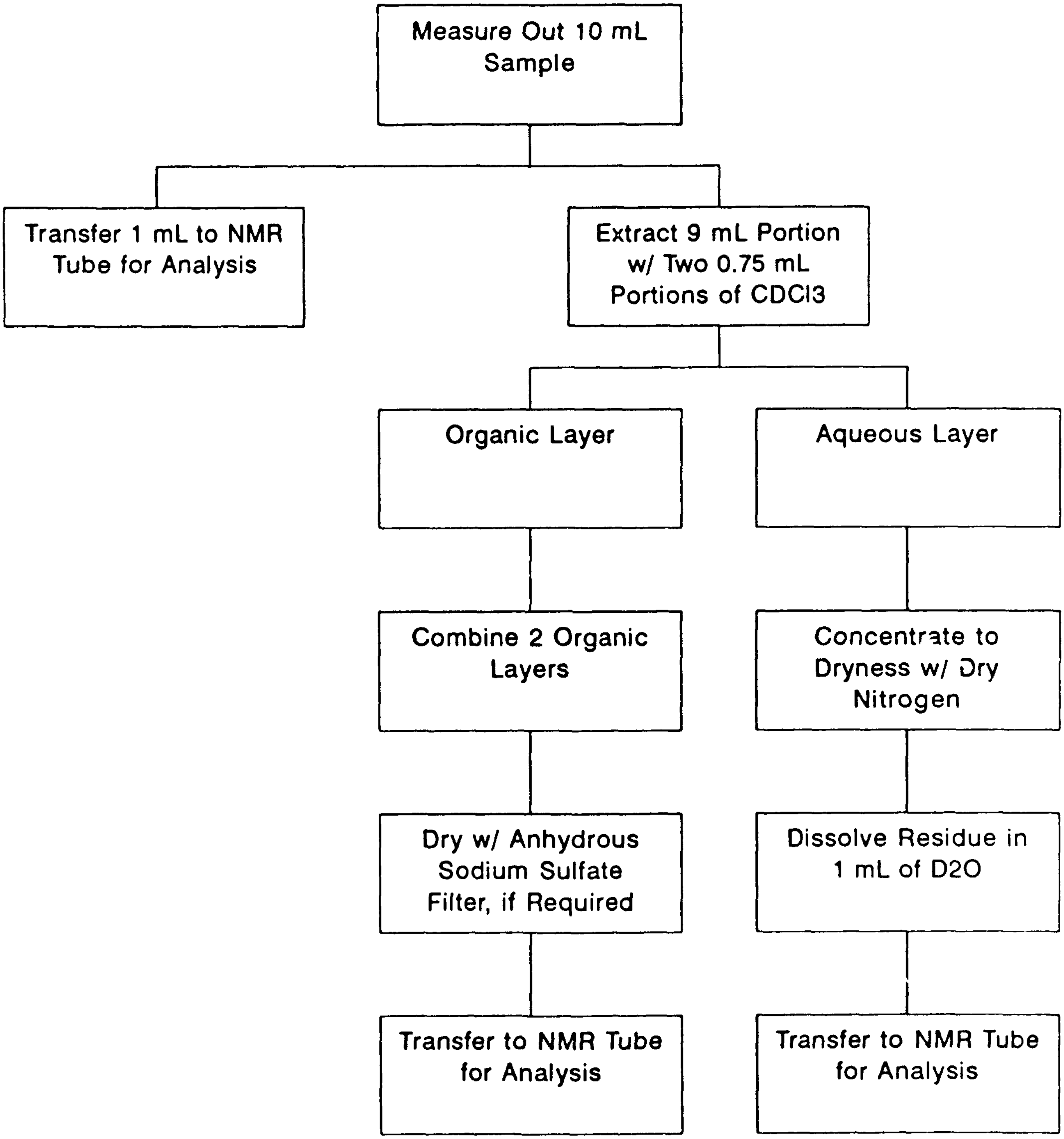
Scheme C-2. Soil Sample Preparation For NMR Analysis (Cont'd)



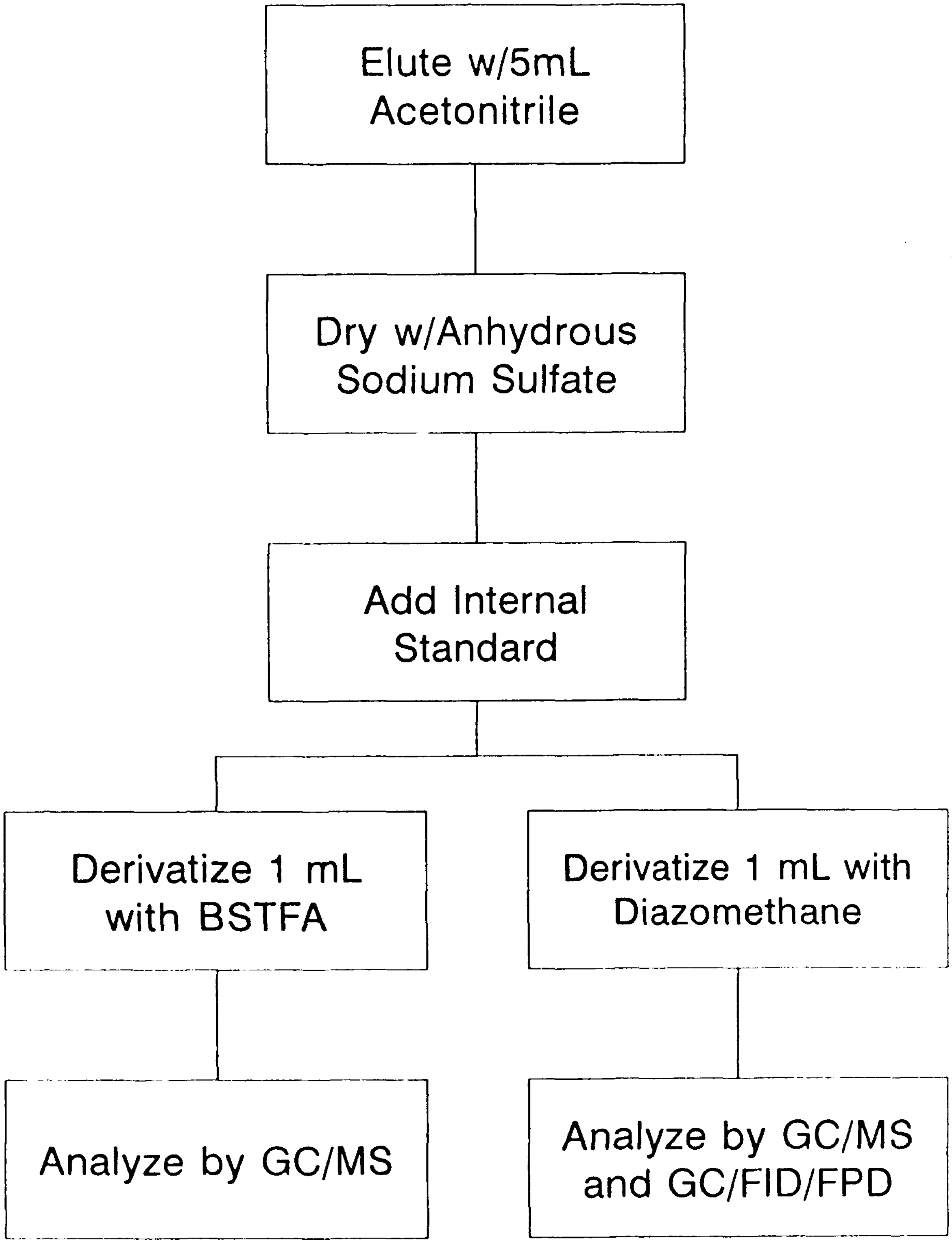
Scheme C-3. Water Sample Preparation For Chromatographic Analysis



Scheme C-4. Water Sample Preparation For NMR Analysis



Scheme C-5. C-18 Cartridge Sample Preparation



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APPENDIX D

NMR METHODS AND SPECTRA

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR SAMPLE IDENTIFICATION

*1.1 NMR sample code:	1.2 Sample preparation date:
W15-C	9 March 1993

SOLVENT

*1.3 Name and/or formula:	1.4 Deuteration degree or purity:	1.5 Manufacturer:
D_2O	99.9 atom % D	MSD Isotopes

CHEMICAL SHIFT REFERENCE COMPOUND(S)

*1.6 For nucleus	*1.7 Name and/or formula	*1.8 Type	1.9 Manufacturer	1.10 Purity
^{1}H, ^{13}C	Sodium 3-trimethylsilyl-propionate-2,2,3,3-d_4 (TSP)	Internal	Merck & Co., Inc.	-
^{31}P	Phosphoric Acid	External	Fisher Sci. Co.	85%

1.11 Preparation of chemical shift reference solution(s):

References were used as received.

NMR TUBE

1.12 Sealed by:	1.13 Outside diameter:	1.14 Tube manufacturer, and tube code:	1.15 Filtration method:
i) melting ii) a cap + Parafilm iii) other means, describe	5-mm	Wilmad 507-PP	i) no filtration ii) through piece of cotton wool iii) through a sintered glass iv) other, describe

SAMPLE PREPARATION

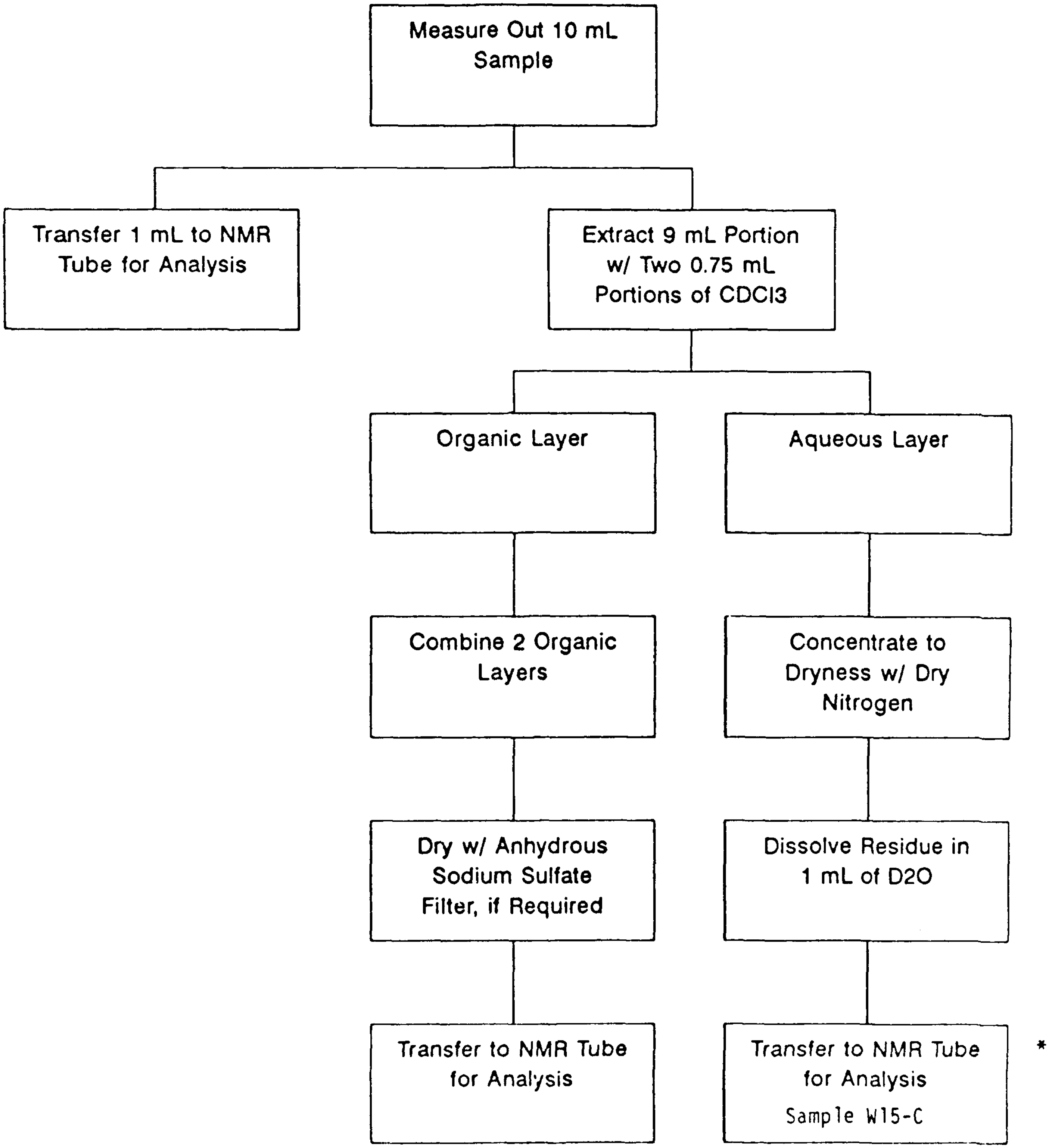
*1.16 Sample preparation details:

After extraction with $CDCl_3$, the aqueous layer of a 9-mL portion of the original sample was concentrated to dryness using dry nitrogen. The residue was dissolved in 1-mL of D_2O. This sample was transferred to an NMR tube for analysis.

See attached block diagram.

1.17 Notes

Aqueous Liquid Sample Preparation for NMR Analysis



Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR INSTRUMENTATION			
*2.1 NMR spectrometer: Varian VXR-400S		2.2 Spectrometer manufacturer: Varian Associates	
2.3* Proton frequency [MHz]: 399.95 MHz	2.4 Temperature control unit present: YES / NO	2.5 Install. year: 1988	2.6 Spectrometer operating system: VNMRS 4.1B
*2.7 Probehead(s)			2.8 Computer operating system: SUNOS 4.1.2
*2.7.1 Probehead name	*2.7.2 Observable nuclei	*2.7.3 Sample diameter	2.9 Notes:
Broadband/switch-able	^{1}H, ^{19}F and ^{15}N thru ^{31}P	5-mm	

TESTING OF INSTRUMENT PERFORMANCE					
*3.1 Nucleus	(3.2) *2.7.1 Probehead name	*3.3 Test date	*3.4 Test name, test sample, and sample origin	*3.5 Result (S/N, linewidth)	*3.6 Specification value
^{1}H	BB/swit.	2 Mar 93	Sensitivity, 0.1% ethyl benzene/$CDCl_3$ (Varian)	130	100
^{19}F	BB/swit.	2 Mar 93	Sensitivity, 0.05% CF_3-C_6H_5 in C_6D_6 (Varian)	215	100
^{13}C	BB/swit.	2 Mar 93	Sensitivity, 40% Dioxane, 60% C_6D_6 (Varian)	165	120
^{31}P	BB/swit.	2 Mar 93	Sensitivity, 0.0485M Tri-phenyl Phosphate/$CDCl_3$ (VA)	167	100
^{13}C	BB/swit.	16 Dec 92	Resolution, 40% Dioxane, 60% C_6D_6 (Varian)	0.15	$\leq$0.2

3.7 Notes: S/N measurement used as criteria for instrument performance since S/N specification will not be met unless resolution and pulse width are within specifications.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*4.1 Method: 1H NMR 13C-{1H} NMR 19F NMR 31P-{1H} NMR 31P NMR other, describe:

*4.2 Spectrum number: NMR-1	(4.3) *1.1 NMR sample code: W15-C	(4.4) *2.1 NMR spectrometer: Varian VXR-400S	4.5 Recording date: 10 March 1993
*4.6 Observation frequency [MHz]: 399.952	*4.7 Sample temperature [°C]: +22 °C Controlled: YES NO	*4.8 Spectral width [Hz]: 8000	4.9 Spectral width [ppm]: 20 ppm
*4.10 Obs. pulse angle (degrees): 36°	*4.11 Obs. pulse duration (µs): 12.0	4.12 Pulse sequence name: S2PUL	*4.13 Number of scans: 768
*4.14 Repetition time (s): 5.0	4.15 Total acquisition time: 64 min	*4.16 Number od data points in FID: 48,000	*4.17 Number of data points in real part of spectrum: 24,000
*4.18 Lock conditions: i) lock provided by solvent ii) experiment without lock iii) other lock system, describe	*4.19 $\nu_{(1/2)}$ and S/N: $\nu_{(1/2)}$= 0.8 Hz of line at 4.82 ppm S/N= 11/1 of line at 3.70 ppm	(4.20) *2.7.1 Probehead name: BB/Switchable	*4.21 Chemical shift reference value [ppm]: 0.0000

4.22 Quantitation A standard solution containing each of the four identified compounds was prepared in D_2O (500 ug/ml each of methylphosphonic acid, diisopropylaminoethanol and benzilic acid and 750 ug/ml 3-quinuclidinol). The ^1H NMR spectrum of this sample was obtained using a 60° pulse width and a repetition rate of 63 sec (all other parameters as above). The ^1H NMR spectrum of W15-C was re-run using the exact same conditions. The absolute intensity integrals of the two spectra were compared to calculate a "ball

4.23 Notes: park" concentration for each component in the aqueous sample.

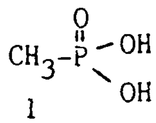
5. Analysis Results

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

Methylphosphonic Acid

*5.2 Molecular structure:
(with numbering of atoms)



1

*5.3 CAS No: 993-13-5

(5.4) *4.2 Spectrum number: NMR-1

(5.5) *1.1 NMR sample code: W15-C

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{1}H NMR

5.8 Analysis date: 10 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	1.09	15.6 (P-H)

5.10 Notes:

Quantitation against a standard solution containing a known amount of the compound indicated the concentration of methylphosphonic acid is ca. 9 ug/ml in the original W15 sample.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:			*5.2 Molecular structure: (with numbering of atoms)
2-(Diisopropylamino)ethanol			$[(CH_3)_2CH]_2N\text{-}CH_2CH_2\text{-}OH$ 1 2 3 4
*5.3 CAS No: 96-80-0	(5.4) *4.2 Spectrum number: NMR-1	(5.5) *1.1 NMR sample code: W15-C	
*5.6 Criteria for positive identification: i) interpretation ii) comparison to a database spectrum iii) comparison to a reference spectrum of authentic compound			(5.7) *4.1 Method: ^1H NMR 5.8 Analysis date: 10 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	1.33	6.4 (H-H), doublet
2	3.70	6.4 (H-H), septet
3	2.9-3.2	Not observed due to peak overlap.
4	3.86	ca. 5.8 (H-H), triplet

5.10 Notes:

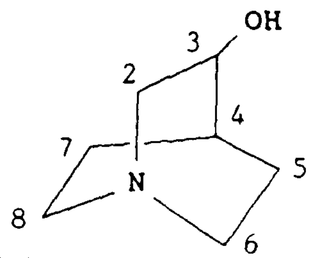
Quantitation against a standard solution containing a known amount of the authentic compound and comparison with a spectrum of W15 before any extractions indicated that the concentration of 2-diisopropylamino-ethanol is ca. 8 ug/ml in the original W15 sample.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

3-Quinuclidinol

*5.2 Molecular structure:
(with numbering of atoms)



*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
1619-34-7	NMR-1	W15-C

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^1H NMR

5.8 Analysis date: 10 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
2a,b	2.96,3.50	
3	4.23	
4	2.17	
5a,b	1.79, 2.12	
6a,b	3.21	
7a,b	1.79, 1.98	
8a,b	3.08, 3.15	

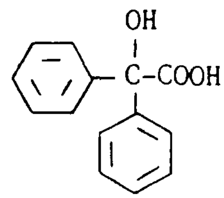
5.10 Notes: Quantitation against a standard solution containing a known amount of the authentic compound indicated the concentration of 3-quinuclidinol is ca. 18 ug/ml in the original W15 solution.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

Benzilic Acid

*5.2 Molecular structure:
(with numbering of atoms)



*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
76-93-7	NMR-1	W15-C

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{1}H NMR

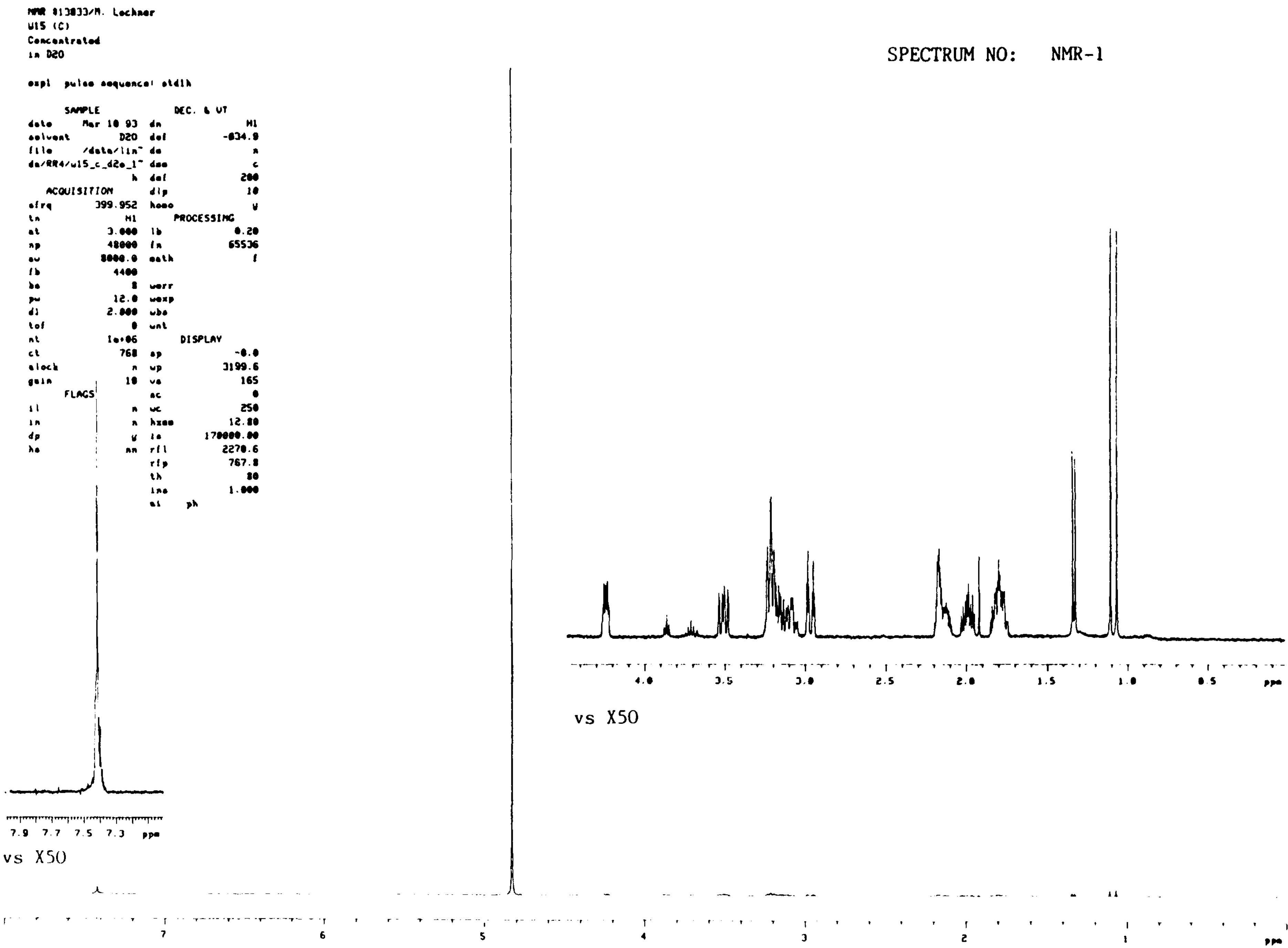
5.8 Analysis date: 10 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
All aromatic protons	7.38-7.44	Not determined.

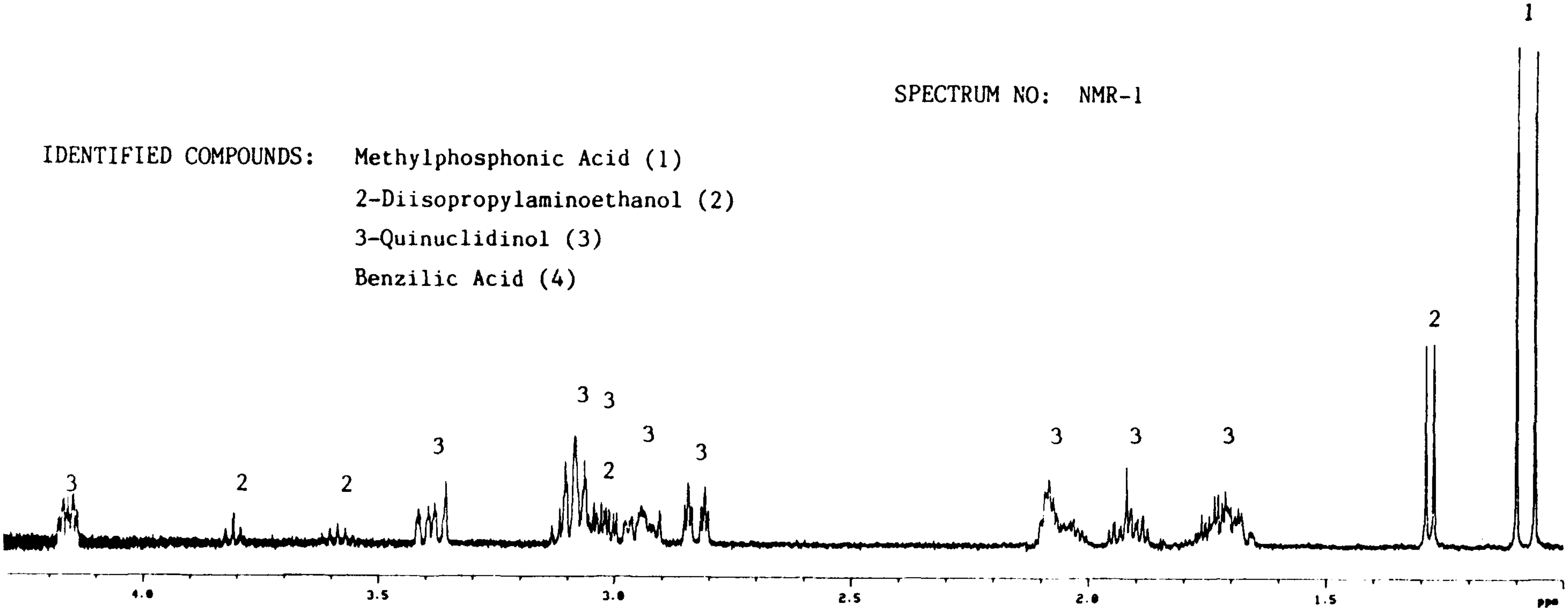
5.10 Notes: Identification made based on pattern observed for both the standard solution and the unknown solution.
Quantitation against a standard solution containing a known amount of the authentic compound indicated the concentration of benzilic acid is ca. 8 ug/ml in the original W15 sample.

SPECTRUM NO: NMR-1

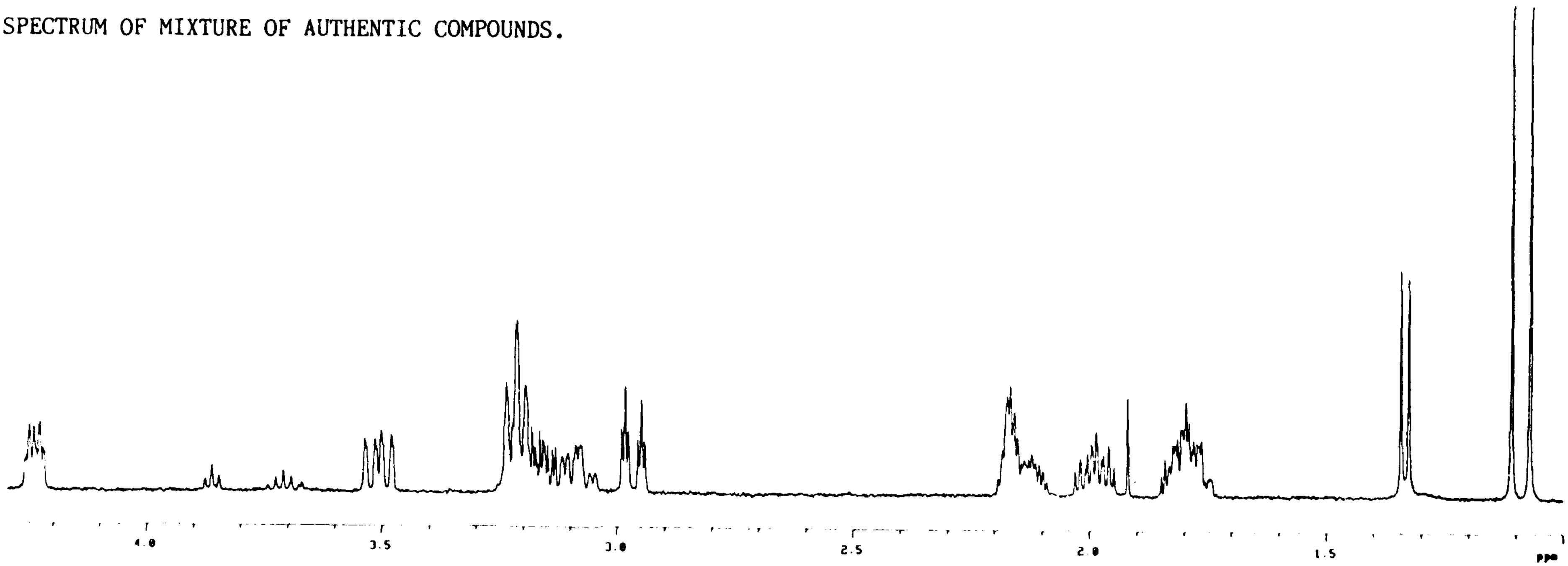


SPECTRUM NO: NMR-1

IDENTIFIED COMPOUNDS: Methylphosphonic Acid (1)
2-Diisopropylaminoethanol (2)
3-Quinuclidinol (3)
Benzilic Acid (4)

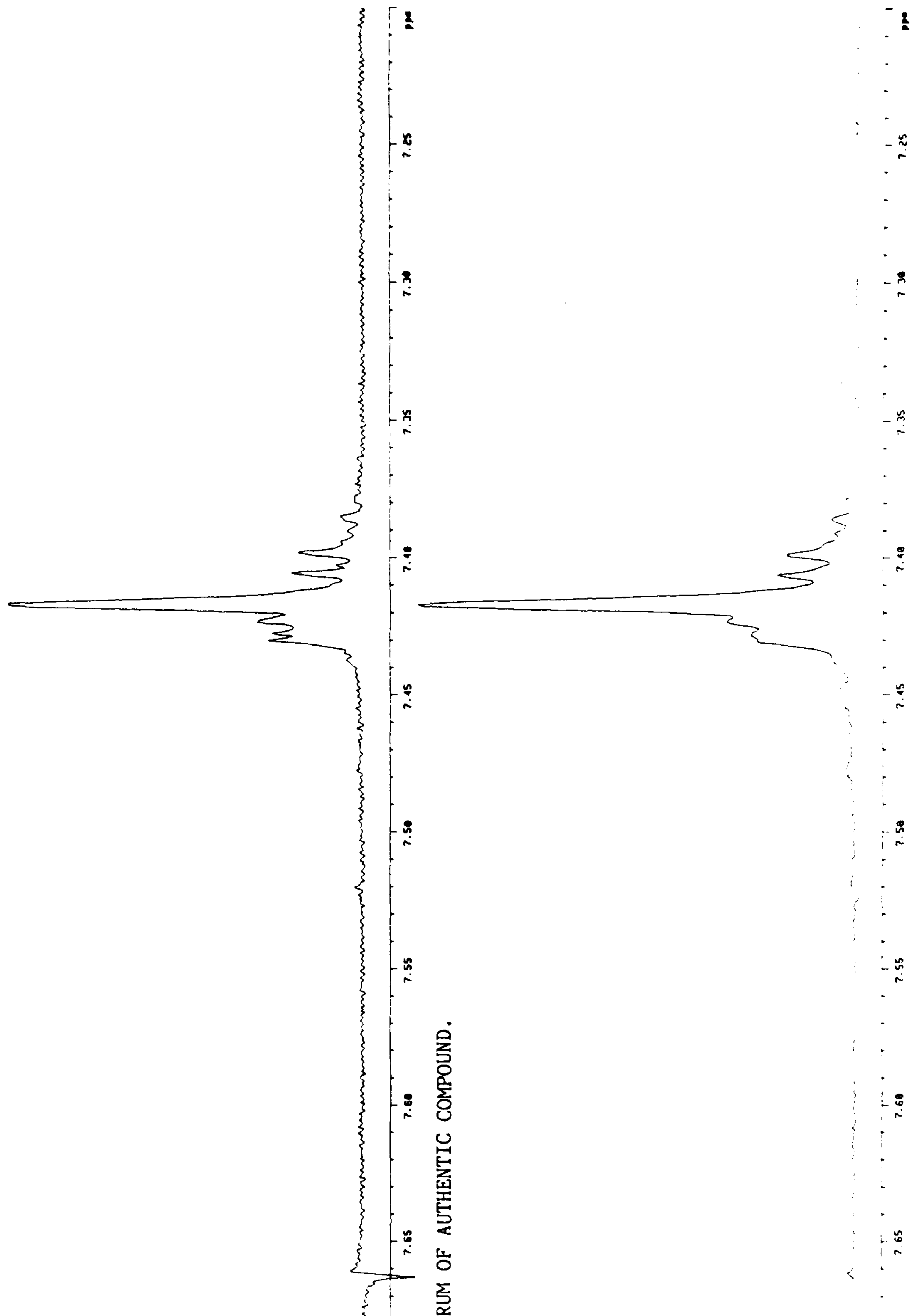


SPECTRUM OF MIXTURE OF AUTHENTIC COMPOUNDS.



SPECTRUM OF SAMPLE W15-C.

SPECTRUM NO: NMR-1



SPECTRUM OF AUTHENTIC COMPOUND.

SPECTRUM OF SAMPLE W15-C.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*4.1 Method: 1H NMR 13C-{1H} NMR 19F NMR 31P-{1H} NMR 31P NMR other, describe:

*4.2 Spectrum number: NMR-2	(4.3) *1.1 NMR sample code: W15-C	(4.4) *2.1 NMR spectrometer: Varian VXR-400S	4.5 Recording date: 11 March 1993
*4.6 Observation frequency [MHz]: 161.903	*4.7 Sample temperature [°C]: +22 °C Controlled: YES NO	*4.8 Spectral width [Hz]: 40,000	4.9 Spectral width [ppm]: 247 ppm
*4.10 Obs. pulse angle (degrees): 47°	*4.11 Obs. pulse duration (μs): 7.3	4.12 Pulse sequence name: S2PUL	*4.13 Number of scans: 1168
*4.14 Repetition time (s): 1.8	4.15 Total acquisition time: 35 min	*4.16 Number od data points in FID: 64,000	*4.17 Number of data points in real part of spectrum: 32,000
*4.18 Lock conditions: i) lock provided by solvent ii) experiment without lock iii) other lock system, describe	*4.19 $\nu_{(1/2)}$ and S/N: $\nu_{(1/2)}$= 1.7 Hz of line at 21.35 ppm S/N= 16/1 of line at 21.35 ppm	(4.20) *2.7.1 Probehead name: BB/Switchable	*4.21 Chemical shift reference value [ppm]: 0.00

4.22 Quantitation

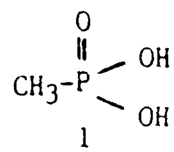
4.23 Notes:

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

Methylphosphonic Acid

*5.2 Molecular structure:
(with numbering of atoms)



*5.3 CAS No: 993-13-5

(5.4) *4.2 Spectrum number: NMR-2

(5.5) *1.1 NMR sample code: W15-C

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{31}P NMR

5.8 Analysis date: 11 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	21.4	15.4 (P-H), quartet

5.10 Notes:

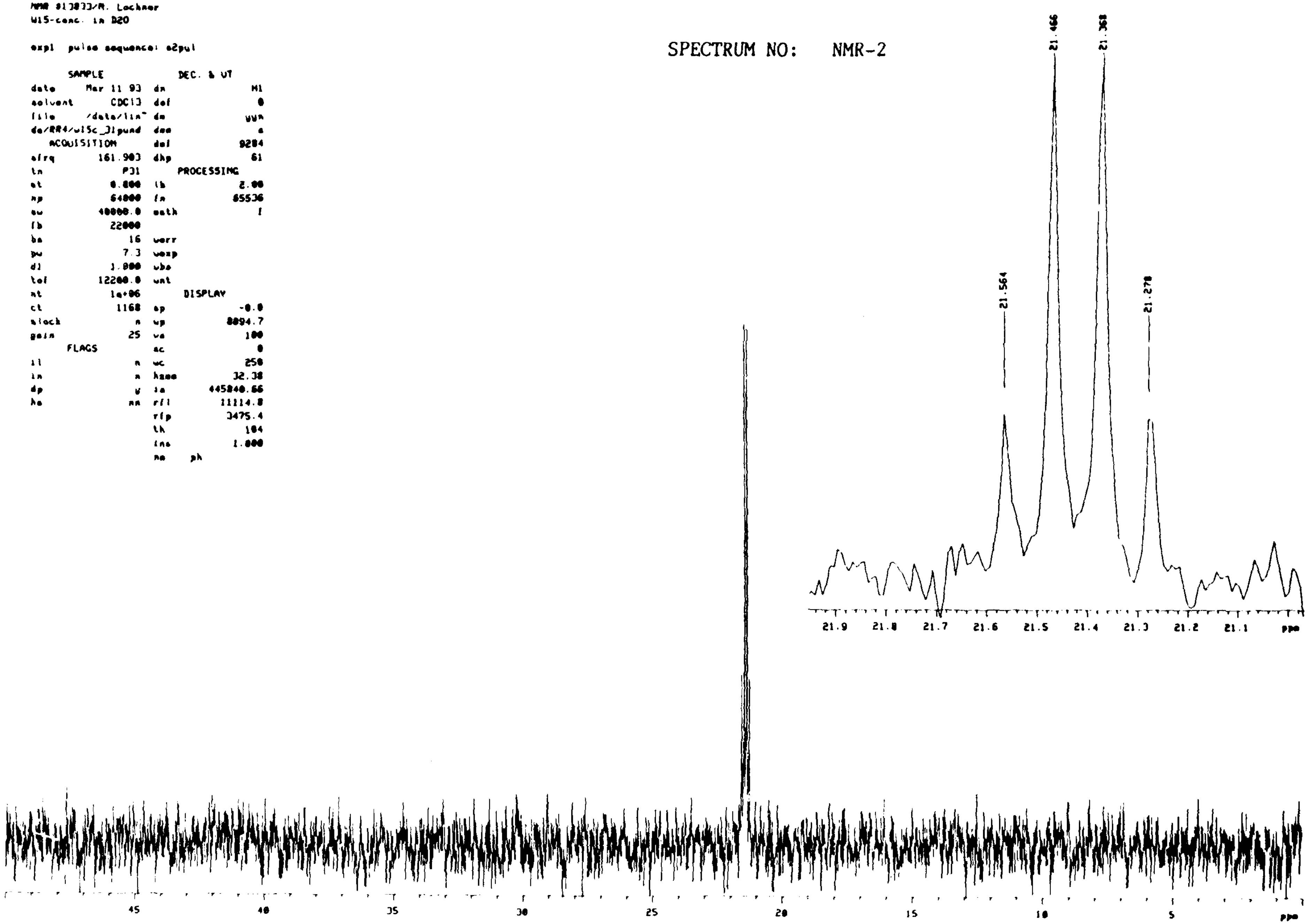
APPENDIX D

NMR #13833/R. Lockner
W15-conc. in D20

exp1 pulse sequence: s2pul

SAMPLE		DEC. & VT	
date	Mar 11 93	dn	H1
solvent	CDCl3	dof	0
file	/data/lin~	dm	yyn
da/RR4/w15c_31pund		dmm	a
ACQUISITION		dmf	9284
sfrq	161.903	dhp	61
tn	P31	PROCESSING	
at	0.800	lb	2.00
np	64000	fn	65536
sw	40000.0	math	f
fb	22000		
bs	16	werr	
pw	7.3	wexp	
d1	1.000	wbs	
tof	12200.0	wnt	
nt	1e+06	DISPLAY	
ct	1168	sp	-0.0
alock	n	wp	8094.7
gain	25	vs	100
FLAGS		sc	0
il	n	wc	250
in	n	hzmm	32.38
dp	y	is	445840.66
hs	nn	rfl	11114.8
		rfp	3475.4
		th	104
		ins	1.000
		nm	ph

SPECTRUM NO: NMR-2



Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

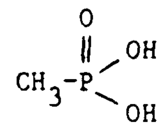
*4.1 Method: 1H NMR 13C-{1H} NMR 19F NMR 31P-{1H} NMR 31P NMR other, describe:			
*4.2 Spectrum number: NMR-3	(4.3) *1.1 NMR sample code: W15-C	(4.4) *2.1 NMR spectrometer: Varian VXR-400S	4.5 Recording date: 12 March 1993
*4.6 Observation frequency [MHz]: 100.577	*4.7 Sample temperature [°C]: +23 °C Controlled: YES / NO	*4.8 Spectral width [Hz]: 25000	4.9 Spectral width [ppm]: 248.5 ppm
*4.10 Obs. pulse angle (degrees): 66°	*4.11 Obs. pulse duration (μs): 9.5	4.12 Pulse sequence name: S2PUL	*4.13 Number of scans: 42024
*4.14 Repetition time (s): 4.1 sec	4.15 Total acquisition time: 48 hrs	*4.16 Number od data points in FID: 80000	*4.17 Number of data points in real part of spectrum: 40000
*4.18 Lock conditions: i) lock provided by solvent ii) experiment without lock iii) other lock system, describe	*4.19 $\nu_{(1/2)}$ and S/N: $\nu_{(1/2)}$= 1.9 Hz of line at 28.8 ppm S/N= 3.3/1 of line at 16.3 ppm	(4.20) *2.7.1 Probehead name: BB/Switchable	*4.21 Chemical shift reference value [ppm]: 0.00
4.22 Quantitation			
4.23 Notes:			

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

Methylphosphonic Acid

*5.2 Molecular structure:
(with numbering of atoms)



1

*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
993-13-5	NMR-3	W15-C

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{13}C NMR

5.8 Analysis date: 15 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	17.0	131 (P-C)

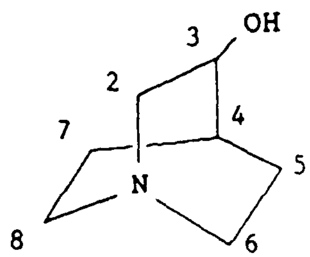
5.10 Notes:

Laboratory/Country: LABORATORY 1? / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

3-Quinuclidinol

*5.2 Molecular structure:
(with numbering of atoms)



*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
1619-34-7	NMR-3	W15-C

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{13}C NMR

5.8 Analysis date: 15 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
2	57.8	
3	67.2	
4	28.8	
5	18.9	
6	49.5	
7	23.3	
8	48.5	

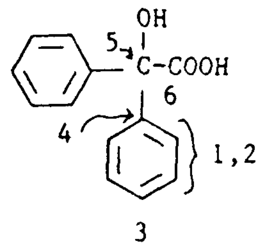
5.10 Notes:

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

Benzilic Acid

*5.2 Molecular structure:
(with numbering of atoms)



*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
76-93-7	NMR-3	W15-C

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{13}C NMR

5.8 Analysis date: 15 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1,2	130.51,131.1	
3	130.56	
4	N.O.	
5	N.O.	
6	N.O.	

5.10 Notes:

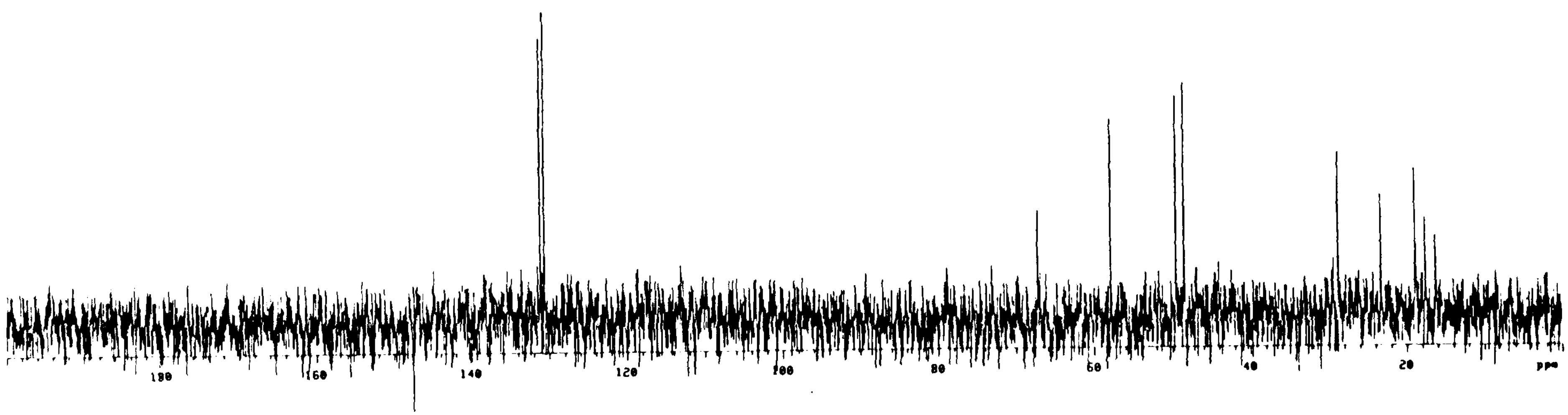
SPECTRUM NO: NMR-3

```
NMR 813835/M. Lochner
W15C
D2O Conc.

exp2  pulse sequence: s2pul

     SAMPLE              DEC. & VT
date     Mar 12 93  dn          H1
solvent        D2O  dof          0
file   /data/lin~   dm         WWW
   da/RR4/w15c_c13  dmm          w
   ACQUISITION      dmf       9284
sfrq       100.577  dhp         61
tn             C13      PROCESSING
at           1.600  lb        3.00
np           80000  lsfid        2
sw         25000.0  fn       65536
fb           13800  math         f
bs              16
pw             9.5  werr
tpwr      not used  wexp
d1           2.500  wbs        wft
tof         1000.0  wnt
nt           1e+06      DISPLAY
ct               0  sp        -0.0
alock            n  wp     20114.7
gain            25  vs          50
     FLAGS          sc           0
il               n  wc         250
in               n  hzmm     80.46
dp               y  is      100.00
hs              nn  rfl     1599.9
                    rfp          0
                    th          12
                    ins      1.000
                    nm    ph
```

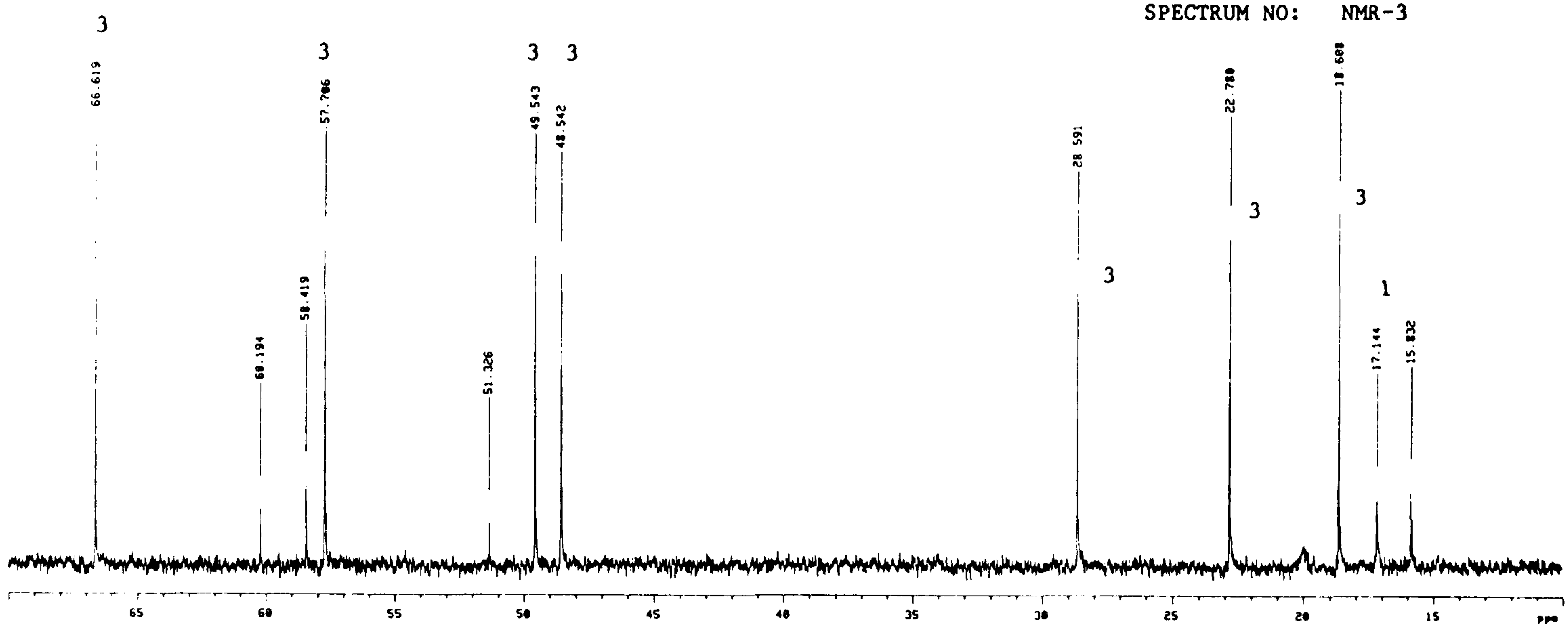
COMPOUNDS IDENTIFIED: Methylphosphonic Acid (1)

3-Quinuclidinol (3)

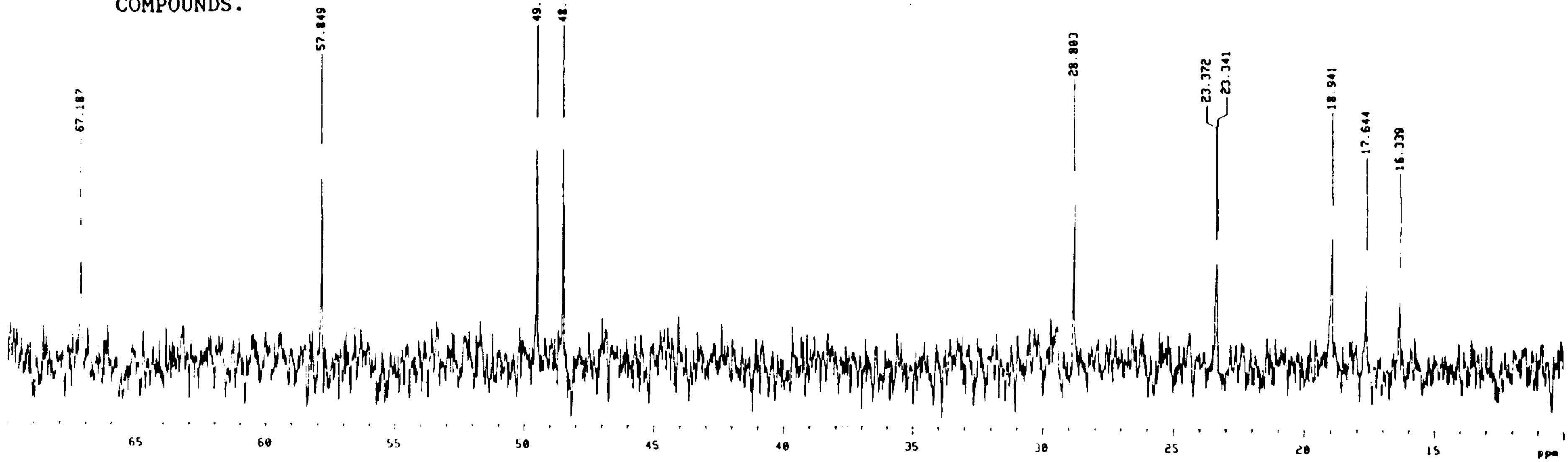
Benzilic Acid (4)



SPECTRUM NO: NMR-3

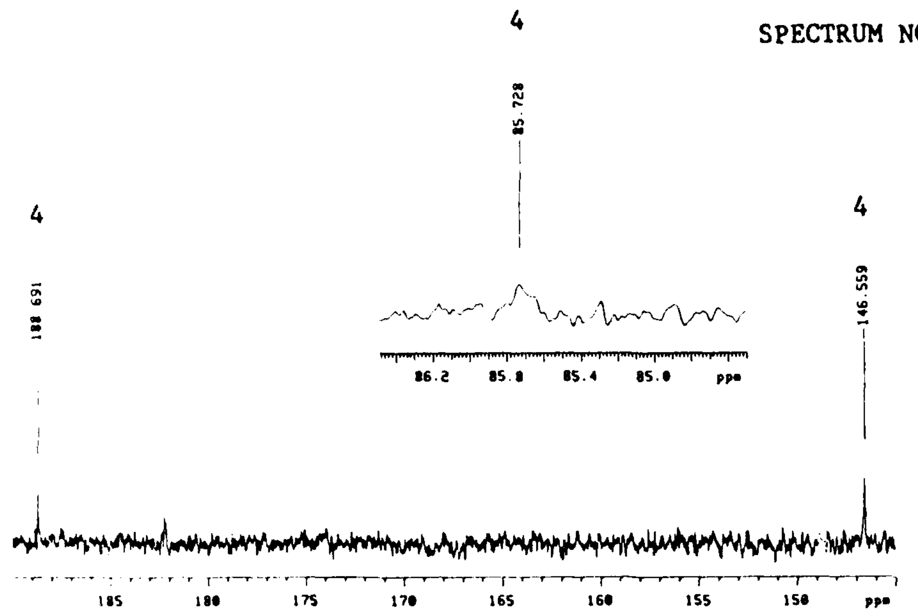


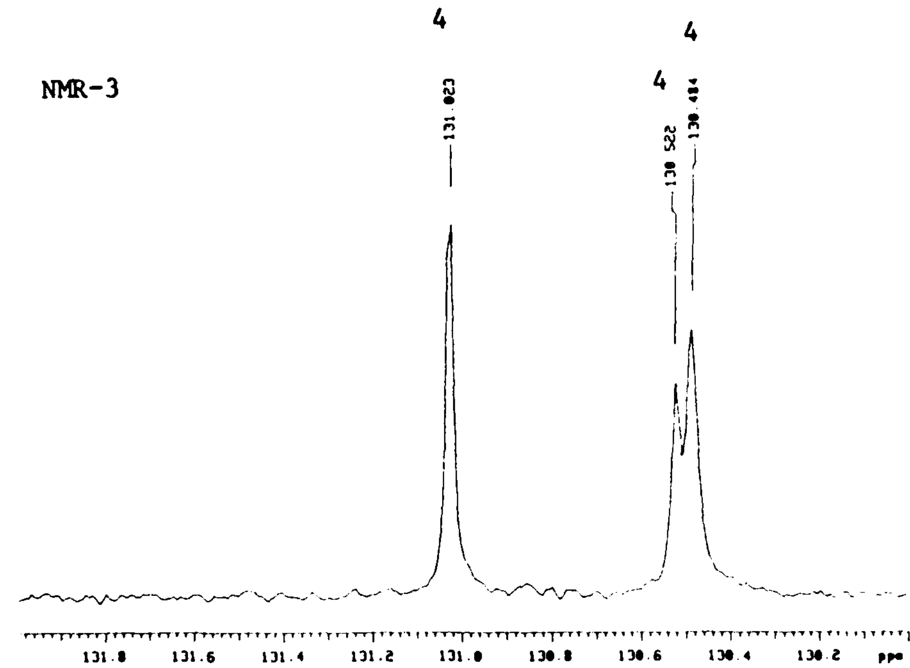
SPECTRUM OF MIXTURE OF AUTHENTIC COMPOUNDS.



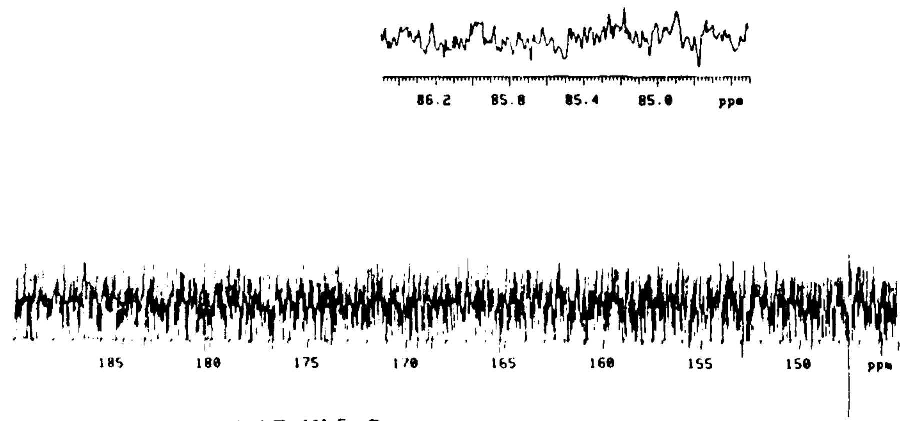
SPECTRUM OF SAMPLE W15-C.

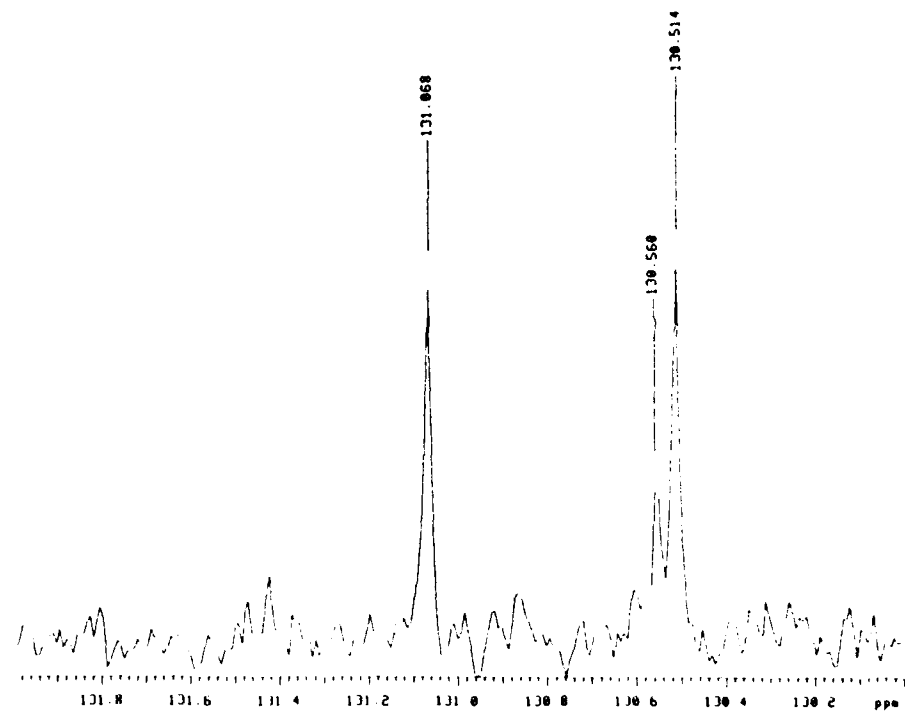
SPECTRUM NO: NMR-3





SPECTRUM OF AUTHENTIC SAMPLE.





SPECTRUM OF SAMPLE W15-C.

1. NMR Sample Preparation

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR SAMPLE IDENTIFICATION

*1.1 NMR sample code: KEW14-C	1.2 Sample preparation date: 9 March 1993

SOLVENT

*1.3 Name and/or formula: D_2O	1.4 Deuteration degree or purity: 99.9 atom % D	1.5 Manufacturer: MSD Isotopes

CHEMICAL SHIFT REFERENCE COMPOUND(S)

*1.6 For nucleus	*1.7 Name and/or formula	*1.8 Type	1.9 Manufacturer	1.10 Purity
^1H	Sodium 3-trimethylsilyl-propionate-2,2,3,3-d_4 (TSP)	Internal	Merck & Co., Inc.	-
^{31}P	Phosphoric Acid	External	Fisher Sci. Co.	85%

1.11 Preparation of chemical shift reference solution(s):

References were used as received.

NMR TUBE

1.12 Sealed by:	1.13 Outside diameter:	1.14 Tube manufacturer, and tube code:	1.15 Filtration method:
i) melting ii) a cap + Parafilm iii) other means, describe	5-mm	Wilmad 507-PP	i) no filtration ii) through piece of cotton wool iii) through a sintered glass iv) other, describe

SAMPLE PREPARATION

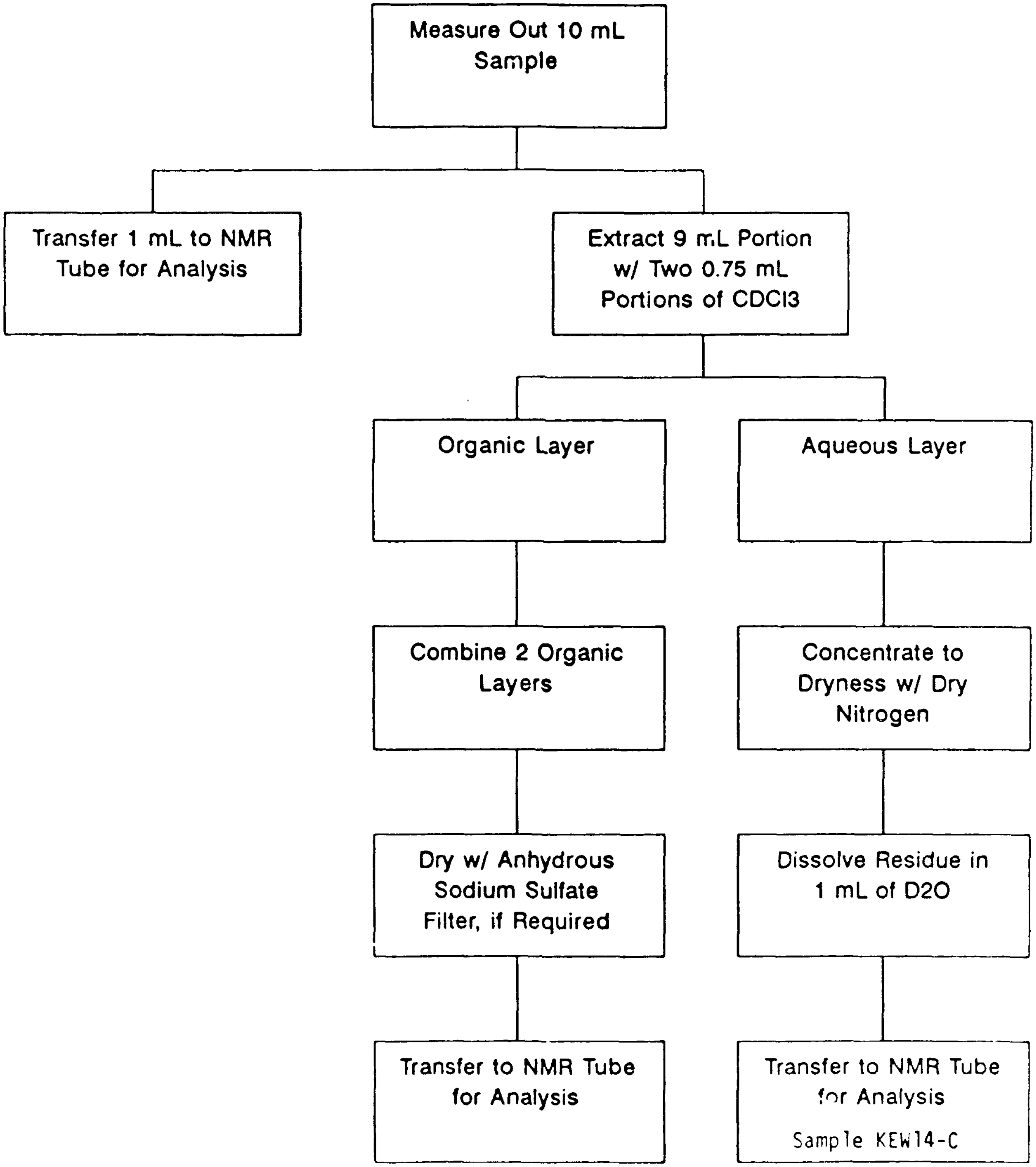
*1.16 Sample preparation details:

After extraction with $CDCl_3$, the aqueous layer of a 9-mL portion of the original sample was concentrated to dryness using dry nitrogen. The residue was dissolved in 1-mL of D_2O. This sample was transferred to an NMR tube for analysis.

See attached block diagram.

1.17 Notes

Aqueous Liquid Sample Preparation for NMR Analysis



*

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR INSTRUMENTATION

*2.1 NMR spectrometer:			2.2 Spectrometer manufacturer:
Varian VXR-400S			Varian Associates
2.3* Proton frequency [MHz]: 399.95 MHz	2.4 Temperature control unit present: YES NO	2.5 Install. year: 1988	2.6 Spectrometer operating system: VNMRS 4.1B
*2.7 Probehead(s)			2.8 Computer operating system: SUNOS 4.1.2
*2.7.1 Probehead name	*2.7.2 Observable nuclei	*2.7.3 Sample diameter	2.9 Notes:
Broadband/switch-able	^{1}H, ^{19}F and ^{15}N thru ^{31}P	5-mm	

TESTING OF INSTRUMENT PERFORMANCE

*3.1 Nucleus	(3.2) *2.7.1 Probehead name	*3.3 Test date	*3.4 Test name, test sample, and sample origin	*3.5 Result (S/N, linewidth)	*3.6 Specification value
^{1}H	BB/swit.	2 Mar 93	Sensitivity, 0.1% ethyl benzene/$CDCl_3$ (Varian)	130	100
^{19}F	BB/swit.	2 Mar 93	Sensitivity, 0.05% CF_3-C_6H_5 in C_6D_6 (Varian)	215	100
^{13}C	BB/swit.	2 Mar 93	Sensitivity, 40% Dioxane, 60% C_6D_6 (Varian)	165	120
^{31}P	BB/swit.	2 Mar 93	Sensitivity, 0.0485M Tri-phenyl Phosphate/$CDCl_3$ (VA)	167	100
^{13}C	BB/swit.	16 Dec 92	Resolution,40% Dioxane, 60% C_6D_6 (Varian)	0.15	≤ 0.2

3.7 Notes: S/N measurement used as criteria for instrument performance since S/N specification will not be met unless resolution and pulse width are within specifications.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

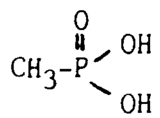
*4.1 Method: 1H NMR 13C-{1H} NMR 19F NMR 31P-{1H} NMR 31P NMR other, describe:

*4.2 Spectrum number: NMR-4	(4.3) *1.1 NMR sample code: KEW14-C	(4.4) *2.1 NMR spectrometer: Varian VXR-400S	4.5 Recording date: 10 March 1993
*4.6 Observation frequency [MHz]: 399.952	*4.7 Sample temperature [°C]: +22 °C Controlled: YES NO	*4.8 Spectral width [Hz]: 8000	4.9 Spectral width [ppm]: 20 ppm
*4.10 Obs. pulse angle (degrees): 36^0	*4.11 Obs. pulse duration (µs): 12.0	4.12 Pulse sequence name: S2PUL	*4.13 Number of scans: 232
*4.14 Repetition time (s): 5.0	4.15 Total acquisition time: 19.7 min	*4.16 Number od data points in FID: 48,000	*4.17 Number of data points in real part of spectrum: 24,000
*4.18 Lock conditions: i) lock provided by solvent ii) experiment without lock iii) other lock system, describe	*4.19 $\nu_{(1/2)}$ and S/N: $\nu_{(1/2)}$= 0.70 Hz of line at 4.82 ppm S/N= 3/1 of line at 3.70 ppm	(4.20) *2.7.1 Probehead name: BB/Switchable	*4.21 Chemical shift reference value [ppm]: 0.0000

4.22 Quantitation A standard solution containing each of the four identified compounds was prepared in D_2O (500 ug/ml each of methylphosphonic acid, diisopropylaminoethanol and benzilic acid, and 750 ug/ml of 3-quinuclidinol). The ^1H NMR spectrum of this sample was obtained using a 60^0 pulse width and a repetition rate of 63 sec (all other parameters as above). The ^1H NMR spectrum of KEW14-C was re-run using the exact same conditions. The absolute intensity integrals of the two spectra were compared to calculate a "ballpark" concentration for each component in the original sample.

4.23 Notes:

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound: Methylphosphonic Acid			*5.2 Molecular structure: (with numbering of atoms)  1
*5.3 CAS No: 993-13-5	(5.4) *4.2 Spectrum number: NMR-4	(5.5) *1.1 NMR sample code: KEW14-C	
*5.6 Criteria for positive identification: i) interpretation ii) comparison to a database spectrum iii) comparison to a reference spectrum of authentic compound			(5.7) *4.1 Method: ^{1}H NMR 5.8 Analysis date: 10 March 1993

***5.9 Interpretation**

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	1.09	15.6 (P-H), doublet

5.10 Notes: Quantitation against a standard solution containing a known amount of the authentic compound indicated that the concentration of methylphosphonic acid is ca. 9 ug/ml in the original KEW14 sample.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:			*5.2 Molecular structure: (with numbering of atoms)
2-(Diisopropylamino)ethanol			$[(CH_3)_2CH]_2N\text{-}CH_2CH_2\text{-}OH$ 1 2 3 4
*5.3 CAS No: 96-80-0	(5.4) *4.2 Spectrum number: NMR-4	(5.5) *1.1 NMR sample code: KEW14-C	
*5.6 Criteria for positive identification: i) interpretation ii) comparison to a database spectrum iii) comparison to a reference spectrum of authentic compound			(5.7) *4.1 Method: ^1H NMR 5.8 Analysis date: 10 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	1.33	6.4 (H-H), doublet
2	3.70	6.4 (H-H), septet
3	2.9-3.2	Not observed due to peak overlap.
4	3.86	ca. 5.8 (H-H), triplet

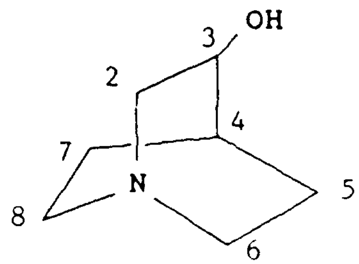
5.10 Notes: Quantitation against a standard solution containing a known amount of the authentic compound indicated that the concentration of the 2-diisopropylaminoethanol is ca. 1 ug/ml in the original KEW14 sample.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

3-Quinuclidinol

*5.2 Molecular structure:
(with numbering of atoms)



*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
1619-34-7	NMR- 4	KEW14-C

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{1}H NMR

5.8 Analysis date: 10 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
2a,b	2.96, 3.50	
3	4.23	
4	2.17	
5a,b	1.79, 2.12	
6a,b	3.20	
7a,b	1.79, 1.98	
8a,b	3.07, 3.14	

5.10 Notes: Quantitation against a standard solution containing a known amount of the authentic compound indicated that the concentration of 3-quinuclidinol is ca. 13 ug/ml in the original KEW14 solution.

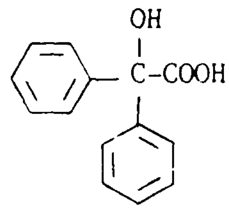
5. Analysis Results

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

Benzilic Acid

*5.2 Molecular structure:
(with numbering of atoms)



*5.3 CAS No: 76-93-7

(5.4) *4.2 Spectrum number: NMR-4

(5.5) *1.1 NMR sample code: KEW-14-C

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{1}H NMR

5.8 Analysis date: 10 March 1993

*5.9 Interpretation

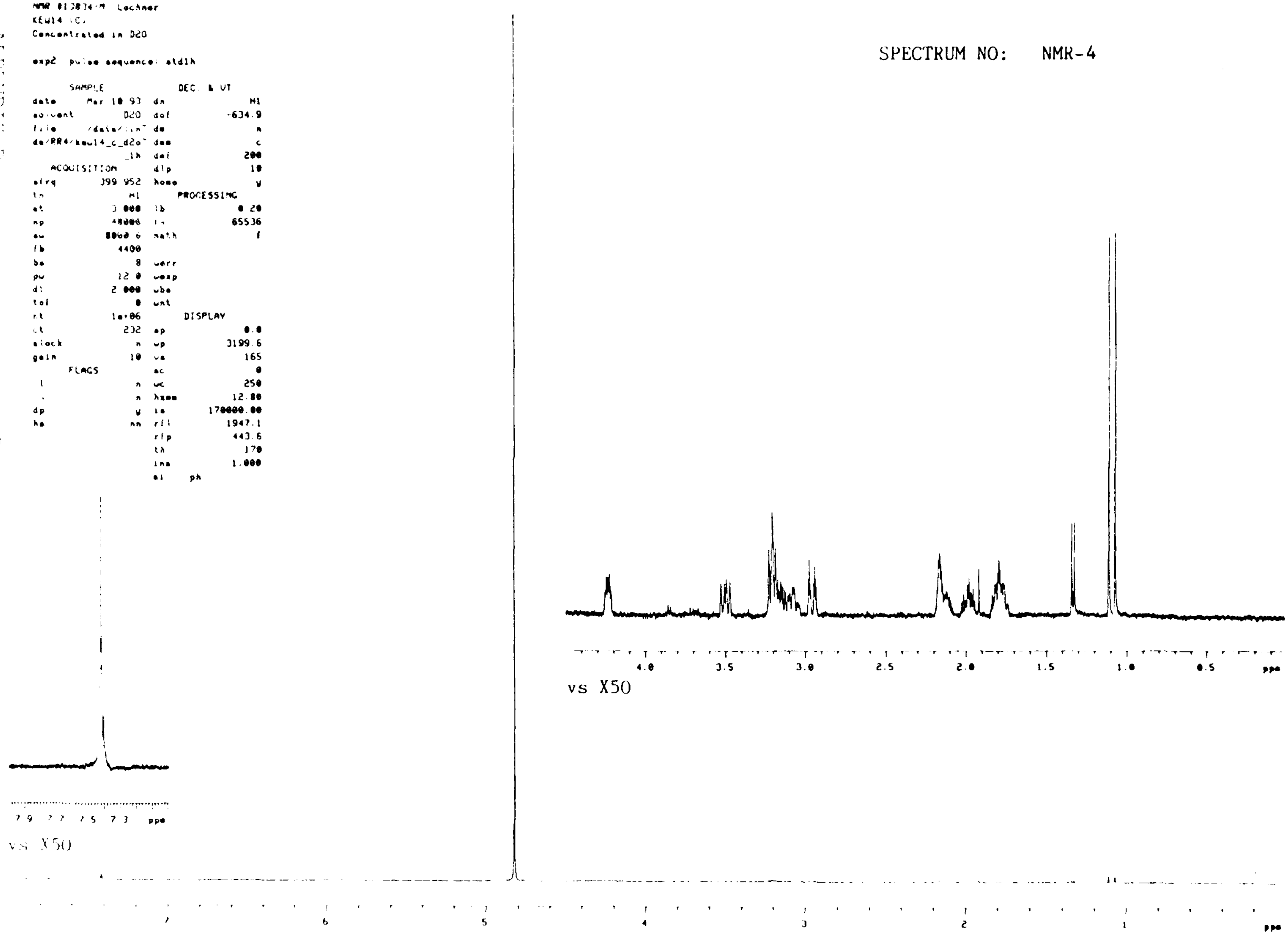
5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
All aromatic protons	7.38-7.44	Not determined.

5.10 Notes: Identification made based on pattern observed for both the standard solution and the unknown solution.

Quantitation against a standard solution containing a known amount of the authentic compound indicated that the concentration of benzilic acid is ca. 7 ug/ml in the original KEW14 sample.

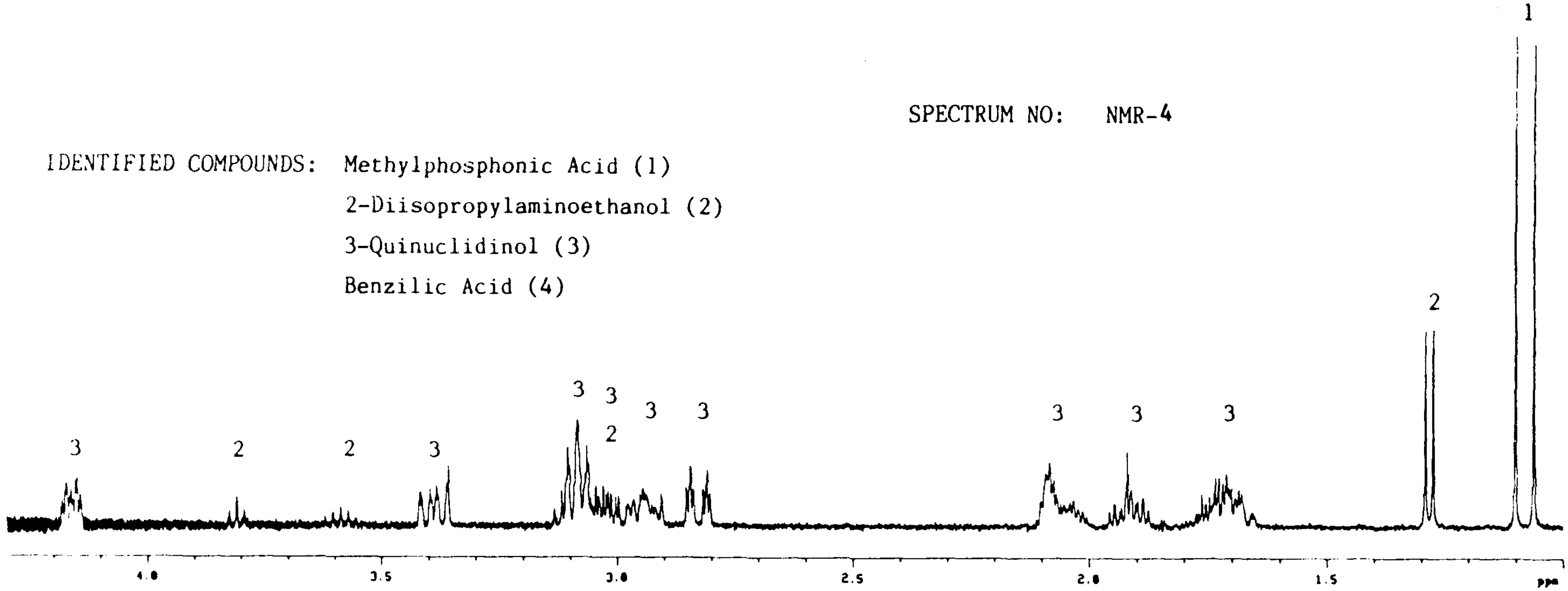
SPECTRUM NO: NMR-4

```
NMR #138344/M Lochner
KEW14 (C)
Concentrated in D2O

exp2  pulse sequence: atd1h

      SAMPLE              DEC. & VT
date     Mar 10 93   dn            H1
solvent        D2O   dof       -634.9
file   /data/lin~    dm             n
de/PR4/kew14_c_d2o~  dmm            c
               _1h   dmf          200
   ACQUISITION       dlp           10
sfrq       399.952   homo           y
tn              H1       PROCESSING
at           3.000   lb          0.20
np           48000   fn         65536
sw          8000.0   math           f
fb            4400
bs               8   werr
pw            12.0   wexp
d1           2.000   wbs
tof              0   wnt
nt           1e+06       DISPLAY
ct             232   sp           0.0
alock            n   wp        3199.6
gain            10   vs           165
      FLAGS          sc             0
il               n   wc           250
in               n   hzmm       12.80
dp               y   is     170000.00
hs              nn   rfl       1947.1
                     rfp        443.6
                     th           170
                     ins        1.000
                     ai    ph
```

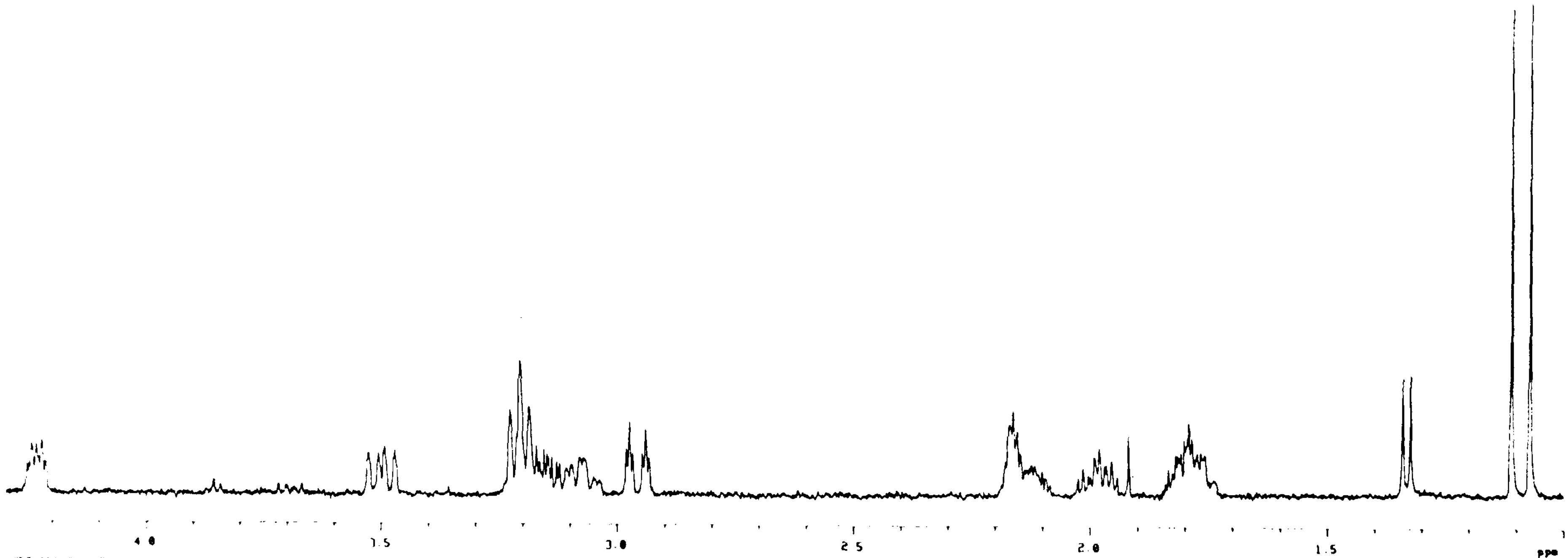


SPECTRUM NO: NMR-4

IDENTIFIED COMPOUNDS: Methylphosphonic Acid (1)
2-Diisopropylaminoethanol (2)
3-Quinuclidinol (3)
Benzilic Acid (4)

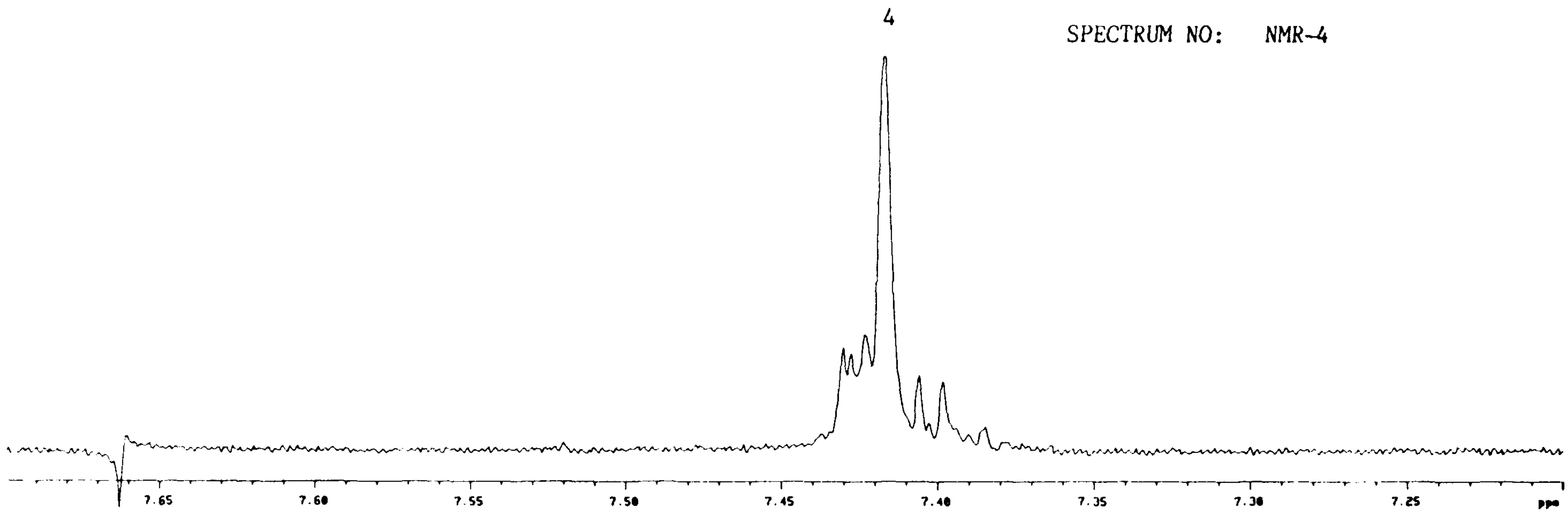


SPECTRUM OF MIXTURE OF AUTHENTIC COMPOUNDS.

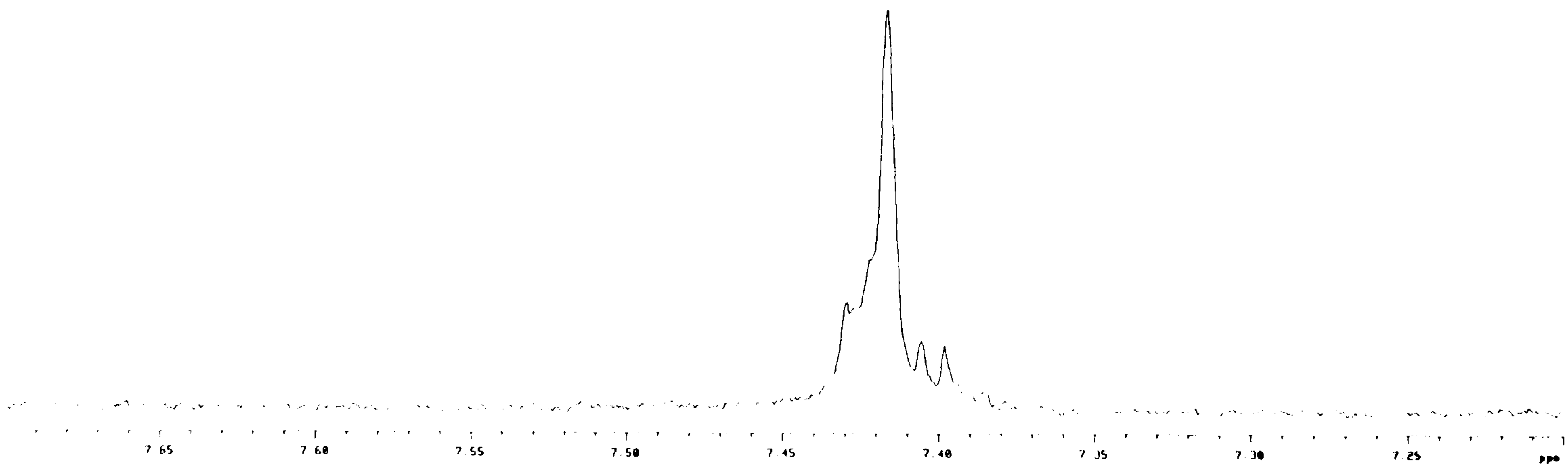


SPECTRUM OF SAMPLE KEW14-C.

SPECTRUM NO: NMR-4



SPECTRUM OF AUTHENTIC COMPOUND.



SPECTRUM OF SAMPLE KEW14-C.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*4.1 Method: 1H NMR 13C-{1H} NMR 19F NMR 31P-{1H} NMR 31P NMR other, describe:			
*4.2 Spectrum number: NMR-5	(4.3) *1.1 NMR sample code: KEW-14C	(4.4) *2.1 NMR spectrometer: Varian VXR-400S	4.5 Recording date: 15 March 1993
*4.6 Observation frequency [MHz]: 161.903	*4.7 Sample temperature [°C]: +22 °C Controlled: YES / NO	*4.8 Spectral width [Hz]: 40,000	4.9 Spectral width [ppm]: 247 ppm
*4.10 Obs. pulse angle (degrees): 47^0	*4.11 Obs. pulse duration (μs): 7.3	4.12 Pulse sequence name: S2PUL	*4.13 Number of scans: 640
*4.14 Repetition time (s): 1.8	4.15 Total acquisition time: 19.3 min	*4.16 Number od data points in FID: 64,000	*4.17 Number of data points in real part of spectrum: 32,000
*4.18 Lock conditions: i) lock provided by solvent ii) experiment without lock iii) other lock system, describe	*4.19 $\nu_{(1/2)}$ and S/N: $\nu_{(1/2)}$= 1.5 Hz of line at 21.38 ppm S/N= 14/1 of line at 21.38 ppm	(4.20) *2.7.1 Probehead name: BB/Switchable	*4.21 Chemical shift reference value [ppm]: 0.00
4.22 Quantitation			
4.23 Notes:			

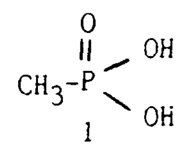
5. Analysis Results

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

Methylphosphonic Acid

*5.2 Molecular structure:
(with numbering of atoms)



*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
993-13-5	NMR- 5	KEW14-C

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{31}P NMR

5.8 Analysis date: 15 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	21.4	15.5 (P-H), quartet

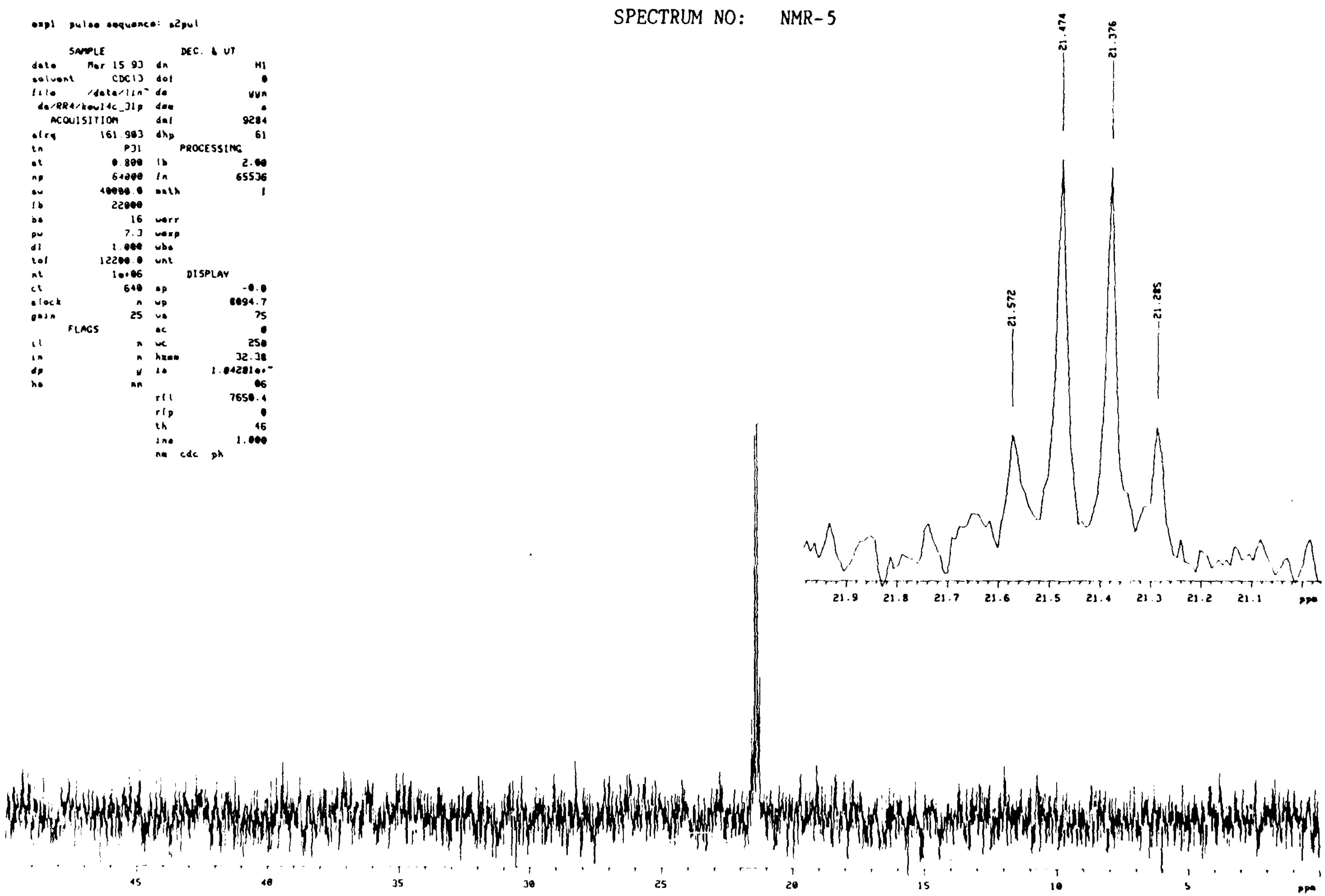
5.10 Notes:

NMR 813834/M. Lochner
KEU14C
D2O xtr

expl pulse sequence: s2pul

SAMPLE		DEC. & VT	
date	Mar 15 93	dn	H1
solvent	CDCl3	dof	0
file	/data/lin~	dm	wyn
da/RR4/keu14c_31p		dmm	a
ACQUISITION		dmf	9284
sfrq	161.983	dhp	61
tn	P31	PROCESSING	
at	0.800	lb	2.00
np	64000	fn	65536
sw	40000.0	math	f
fb	22000		
bs	16	werr	
pw	7.3	wexp	
d1	1.000	wbs	
tof	12200.0	wnt	
nt	1e+06	DISPLAY	
ct	640	sp	-0.0
alock	n	wp	8094.7
gain	25	vs	75
FLAGS		sc	0
il	n	wc	250
in	n	hzmm	32.38
dp	y	is	1.04281e+
hs	nn		06
		rfl	7650.4
		rfp	0
		th	46
		ins	1.000
		nm cdc ph	

SPECTRUM NO: NMR-5



Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR SAMPLE IDENTIFICATION

*1.1 NMR sample code:	1.2 Sample preparation date:
SB15-B	9 March 1993

SOLVENT

*1.3 Name and/or formula:	1.4 Deuteration degree or purity:	1.5 Manufacturer:
D_2O	99.9 atom % D	MSD Isotopes

CHEMICAL SHIFT REFERENCE COMPOUND(S)

*1.6 For nucleus	*1.7 Name and/or formula	*1.8 Type	1.9 Manufacturer	1.10 Purity
^1H	Sodium 3-trimethylsilyl-propionate-2,2,3,3-d_4 (TSP)	Internal	Merck & Co., Inc.	-
^{31}P	Phosphoric Acid	External	Fisher Sci. Co.	85%

1.11 Preparation of chemical shift reference solution(s):

References were used as received.

NMR TUBE

1.12 Sealed by:	1.13 Outside diameter:	1.14 Tube manufacturer, and tube code:	1.15 Filtration method:
i) melting ii) a cap + Parafilm iii) other means, describe	5-mm	Wilmad 507-PP	i) no filtration ii) through piece of cotton wool iii) through a sintered glass iv) other, describe (see 1.16)

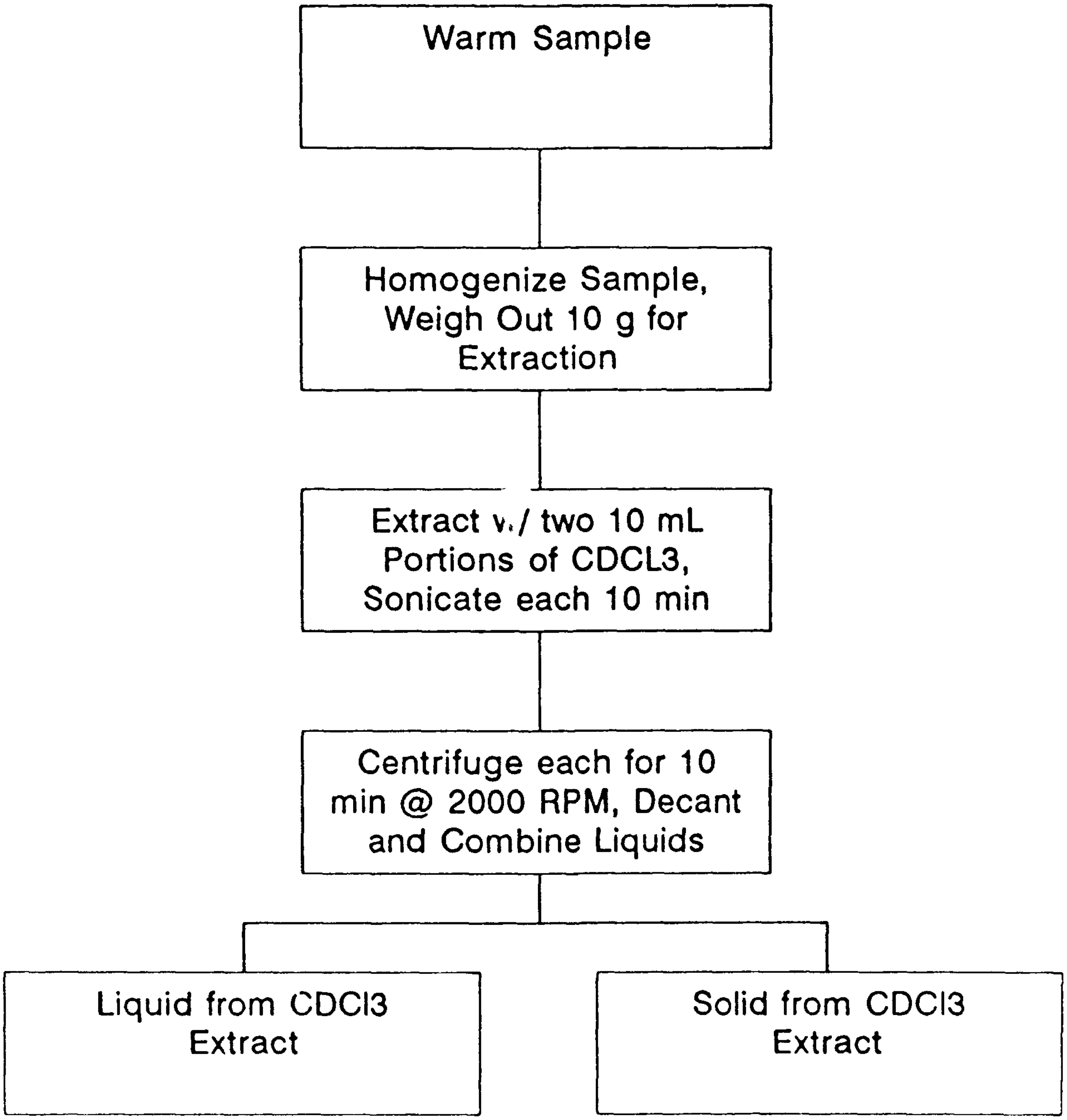
SAMPLE PREPARATION

*1.16 Sample preparation details:

See attached block diagram.

1.17 Notes

Soil Sample Preparation for NMR Analysis



Soil Sample Preparation for NMR Analysis (Continued)

Liquid from CDCl3 Extract

↓

Dry w/ Anhydrous Sodium Sulfate

↓

Filter thru 0.45 μm Disposable Filter

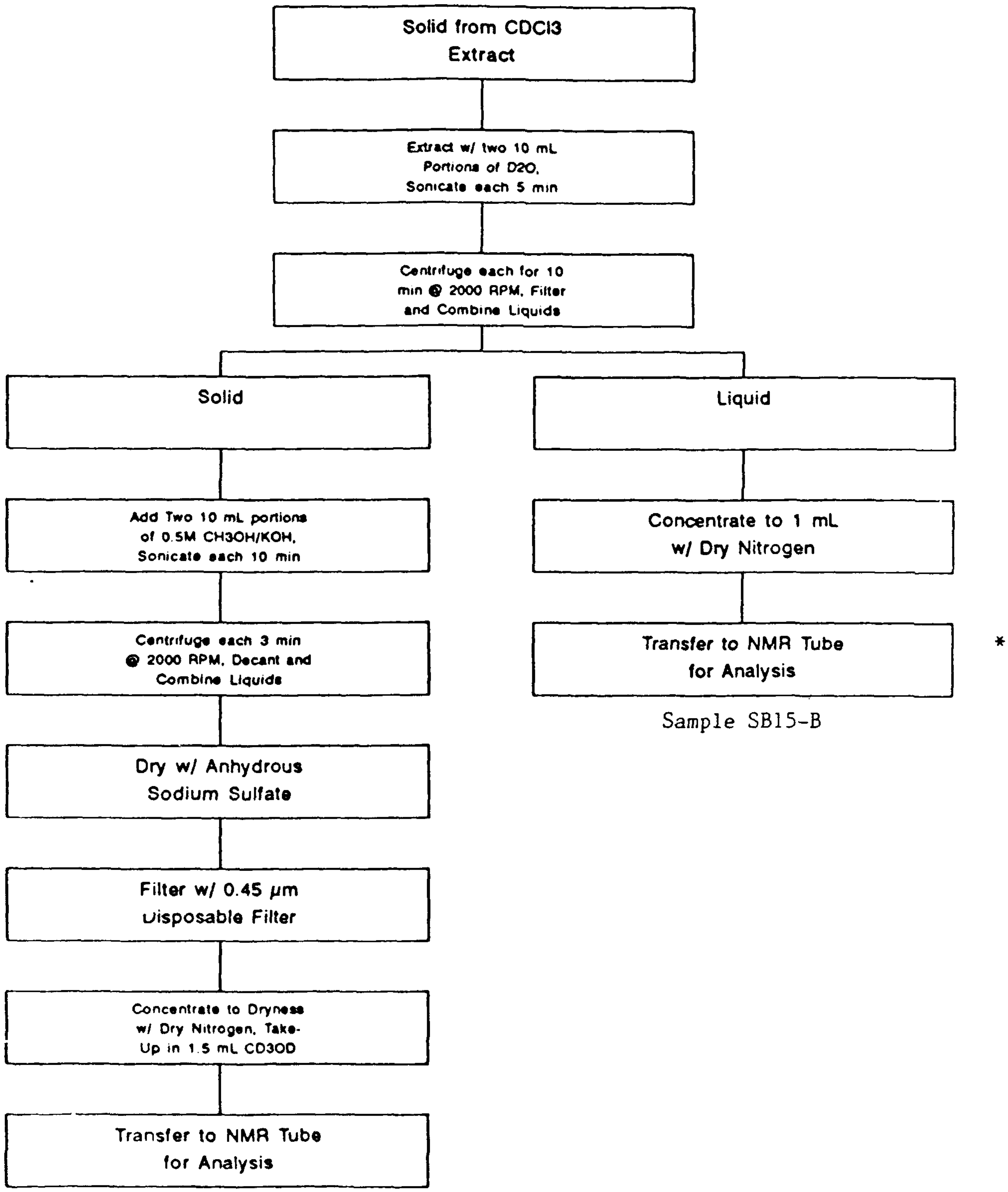
↓

Concentrate to 1 mL w/ Dry Nitrogen

↓

Transfer to NMR Tube for Analysis

Soil Sample Preparation for NMR Analysis (Continued)



Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR INSTRUMENTATION			
*2.1 NMR spectrometer: Varian VXR-400S		2.2 Spectrometer manufacturer: Varian Associates	
2.3* Proton frequency [MHz]: 399.95 MHz	2.4 Temperature control unit present: YES / NO	2.5 Install. year: 1988	2.6 Spectrometer operating system: VNMRS 4.1B
*2.7 Probehead(s)			2.8 Computer operating system: SUNOS 4.1.2
*2.7.1 Probehead name	*2.7.2 Observable nuclei	*2.7.3 Sample diameter	2.9 Notes:
Broadband/switch-able	^1H, ^{19}F and ^{15}N thru ^{31}P	5-mm	

TESTING OF INSTRUMENT PERFORMANCE					
*3.1 Nucleus	(3.2) *2.7.1 Probehead name	*3.3 Test date	*3.4 Test name, test sample, and sample origin	*3.5 Result (S/N, linewidth)	*3.6 Specification value
^1H	BB/swit.	2 Mar 93	Sensitivity, 0.1% ethyl benzene/$CDCl_3$ (Varian)	130	100
^{19}F	BB/swit.	2 Mar 93	Sensitivity, 0.05% CF_3-C_6H_5 in C_6D_6 (Varian)	215	100
^{13}C	BB/swit.	2 Mar 93	Sensitivity, 40% Dioxane, 60% C_6D_6 (Varian)	165	120
^{31}P	BB/swit.	2 Mar 93	Sensitivity, 0.0485M Tri-phenyl Phosphate/$CDCl_3$ (VA)	167	100
^{13}C	BB/swit.	16 Dec 92	Resolution, 40% Dioxane, 60% C_6D_6 (Varian)	0.15	≤ 0.2
3.7 Notes: S/N measurement used as criteria for instrument performance since S/N specification will not be met unless resolution and pulse width are within specifications.					

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

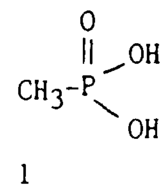
*4.1 Method: 1H NMR 13C-{1H} NMR 19F NMR 31P-{1H} NMR 31P NMR other, describe:			
*4.2 Spectrum number: NMR-6	(4.3) *1.1 NMR sample code: SB15-B	(4.4) *2.1 NMR spectrometer: Varian VXR-400S	4.5 Recording date: 22 March 1993
*4.6 Observation frequency [MHz]: 399.952	*4.7 Sample temperature [°C]: +23 °C Controlled: YES NO	*4.8 Spectral width [Hz]: 8000	4.9 Spectral width [ppm]: 20 ppm
*4.10 Obs. pulse angle (degrees): 45°	*4.11 Obs. pulse duration (µs): 15.0	4.12 Pulse sequence name: S2PUL	*4.13 Number of scans: 8904
*4.14 Repetition time (s): 6.0	4.15 Total acquisition time: 15 hrs	*4.16 Number od data points in FID: 32,000	*4.17 Number of data points in real part of spectrum: 16,000
*4.18 Lock conditions: i) lock provided by solvent ii) experiment without lock iii) other lock system, describe	*4.19 $\nu_{(1/2)}$ and S/N: $\nu_{(1/2)}$= 1.2 Hz of line at 1.37 ppm S/N= 8.4/1 of line at 3.79 ppm	(4.20) *2.7.1 Probehead name: BB/Switchable	*4.21 Chemical shift reference value [ppm]: 0.00
4.22 Quantitation			
4.23 Notes: Continuous homonuclear decoupling used during acquisition. Decoupler frequency set to resonance frequency of the HDO peak. Decoupler gated OFF during acquisition only.			

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

Methylphosphonic Acid

*5.2 Molecular structure:
(with numbering of atoms)



*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
993-13-5	NMR-6	SB15-B

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^1H NMR

5.8 Analysis date: 22 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	1.16	15.6 (P-H), doublet

5.10 Notes:

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

2-Diisopropylamincethanol

*5.2 Molecular structure: (with numbering of atoms)

$[(CH_3)_2CH]_2N-CH_2CH_2-OH$

1 2 3 4

*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
96-80-0	NMR-5	SB15-B

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: 1H NMR

5.8 Analysis date: 22 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	1.37	6.4 (H-H), doublet
2	3.78	6.4 (H-H), septet
3	3.1-3.4	Not observed due to peak overlap.
4	3.89	ca. 5.8, triplet

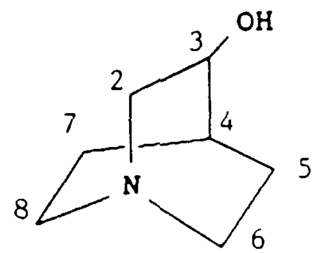
5.10 Notes:

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

3-Quinuclidinol

*5.2 Molecular structure: (with numbering of atoms)



*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
1619-34-7	NMR-6	SB15-B

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^1H NMR

5.8 Analysis date: 22 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
2a,b	3.07, 3.59	
3	N.O.	Not observed due to "ringing" artifacts from large HDO resonance.
4	2.23	
5a,b	1.85, 2.18	
6a,b	3.30	
7a,b	1.85, 2.04	
8a,b	3.24	

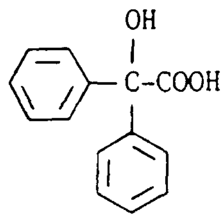
5.10 Notes:

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

Benzilic Acid

*5.2 Molecular structure:
(with numbering of atoms)



*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
76-93-7	NMR-6	SB15-B

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{1}H NMR

5.8 Analysis date: 22 March 1993

*5.9 Interpretation

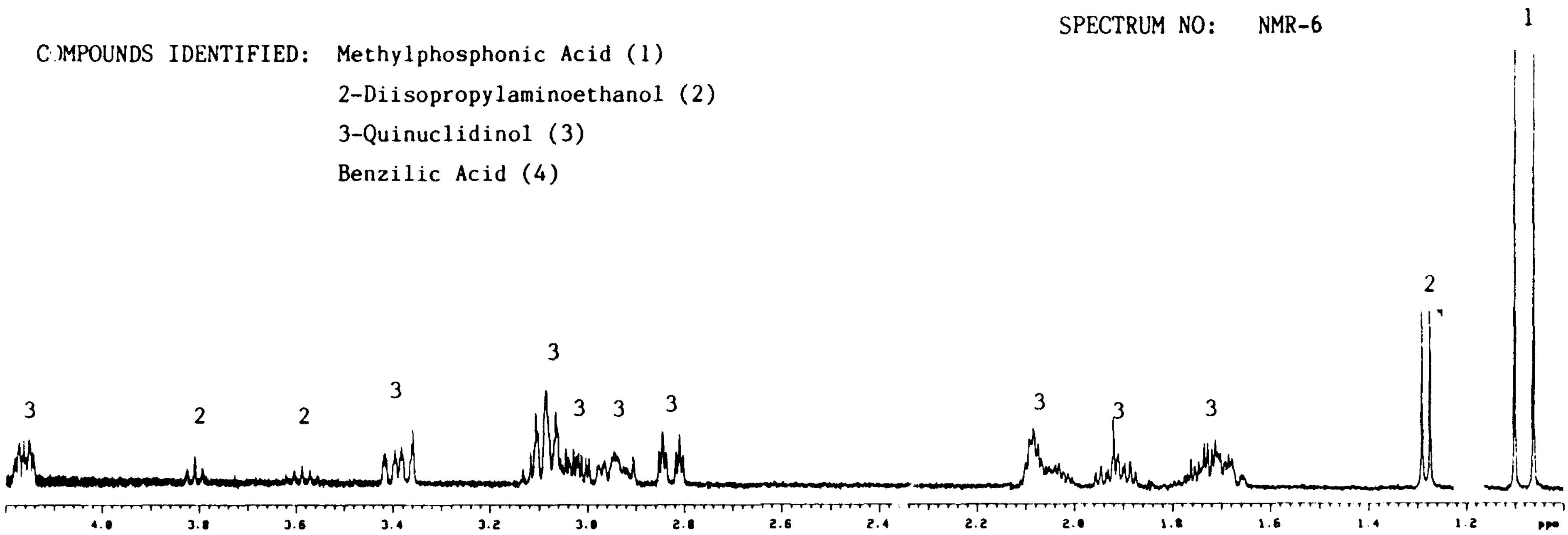
5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
All aromatic protons	7.38-7.45	Not determined.

5.10 Notes:

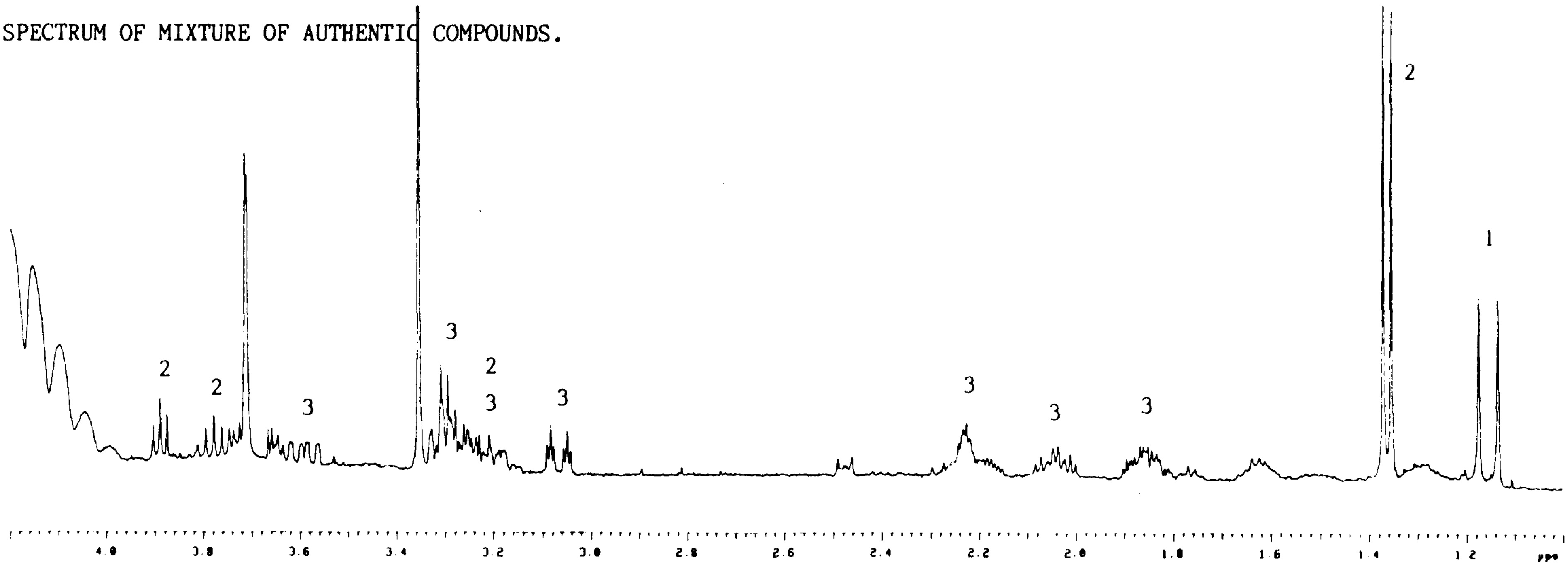
Identification made based on the pattern observed for both the standard solution and the unknown solution.

SPECTRUM NO: NMR-6

COMPOUNDS IDENTIFIED: Methylphosphonic Acid (1)
2-Diisopropylaminoethanol (2)
3-Quinuclidinol (3)
Benzilic Acid (4)



SPECTRUM OF MIXTURE OF AUTHENTIC COMPOUNDS.



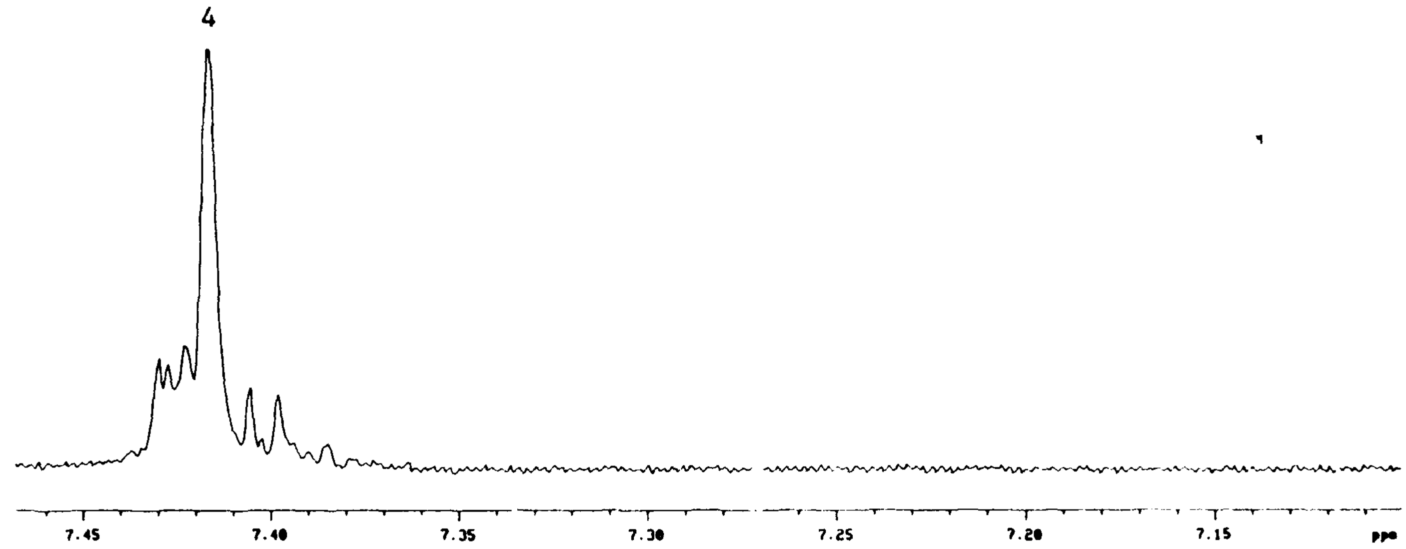
SPECTRUM OF SAMPLE SB15-B.

NMR 813831/M. Lochner
SB15b
D2O xtr

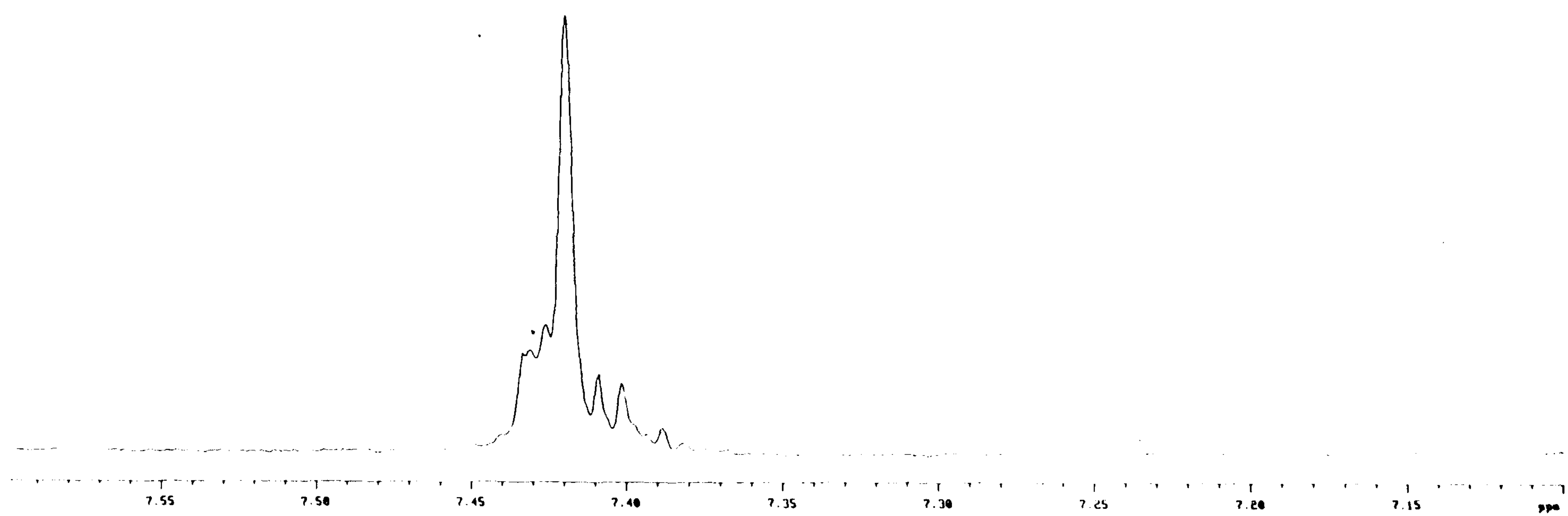
exp1 pulse sequence: std1h

SAMPLE		DEC. & VT	
date	Mar 22 93	dn	H1
solvent	D2O	dof	-568.0
file	exp	dm	yyn
ACQUISITION		dmm	ccc
sfrq	399.952	dmf	200
tn	H1	dlp	5
at	2.000	homo	y
np	32000	temp	23.0
sw	8000.0	PROCESSING	
fb	4400	lb	0.30
bs	8	fn	65536
pw	15.0	math	f
d1	4.000		
tof	-452.3	werr	
nt	1e+06	wexp	
ct	8904	wbs	
alock	n	wnt	
gain	10	DISPLAY	
FLAGS		sp	2839.5
il	n	wp	200.0
in	n	vs	23658
dp	y	sc	0
hs	nn	wc	250
		hzmm	0.80
		is	170000.00
		rfl	2505.4
		rfp	547.9
		th	71
		ins	1.000
		ai	ph

SPECTRUM NO: NMR-6



SPECTRUM OF AUTHENTIC COMPOUND.



SPECTRUM OF SAMPLE SB15-B.

APPENDIX D

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

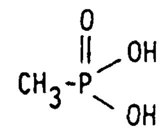
*4.1 Method: 1H NMR 13C-{1H} NMR 19F NMR 31P-{1H} NMR 31P NMR other, describe:			
*4.2 Spectrum number: NMR-7	(4.3) *1.1 NMR sample code: SB15-B	(4.4) *2.1 NMR spectrometer: Varian VXR-400S	4.5 Recording date: 15 March 1993
*4.6 Observation frequency [MHz]: 161.903	*4.7 Sample temperature [°C]: +22 °C Controlled: YES NO	*4.8 Spectral width [Hz]: 40,000	4.9 Spectral width [ppm]: 247 ppm
*4.10 Obs. pulse angle (degrees): 56°	*4.11 Obs. pulse duration (μs): 7.3	4.12 Pulse sequence name: S2PUL	*4.13 Number of scans: 31,552
*4.14 Repetition time (s): 1.8	4.15 Total acquisition time: 16 hrs	*4.16 Number od data points in FID: 64,000	*4.17 Number of data points in real part of spectrum: 32,000
*4.18 Lock conditions: i) lock provided by solvent ii) experiment without lock iii) other lock system, describe	*4.19 $\nu_{(1/2)}$ and S/N: $\nu_{(1/2)}$= 28.5 Hz of line at 22.1 ppm S/N= 5.6 of line at 22.1 ppm	(4.20) *2.7.1 Probehead name: BB/Switchable	*4.21 Chemical shift reference value [ppm]: 0.00
4.22 Quantitation			
4.23 Notes:			

Laboratory/Country: LABORATORY 15 / (ABERDEEN)

*5.1 IUPAC, CA or trivial name of the identified compound:

Methylphosphonic Acid

*5.2 Molecular structure:
(with numbering of atoms)



1

*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
993-13-5	NMR-7	SB15-B

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{31}P NMR

5.8 Analysis date: 15 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	22.1	Not determined.

5.10 Notes:

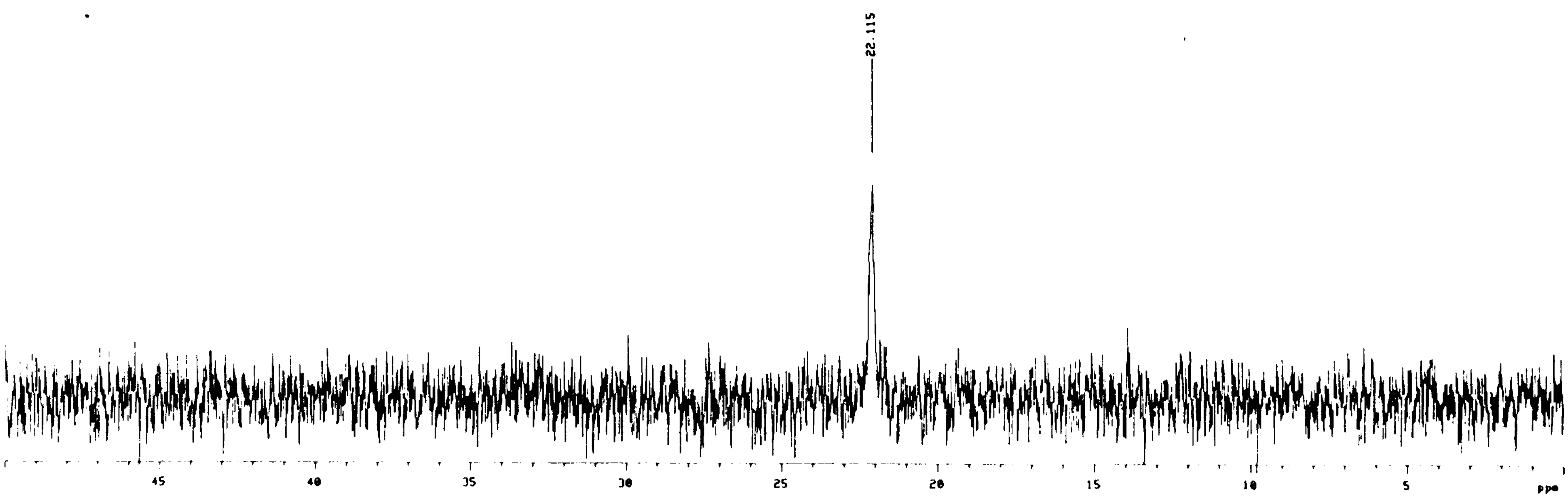
$CH_3\text{-}P(=O)\text{-}$ doublet observed in the ^{1}H NMR spectrum.

SPECTRUM NO: NMR-7

NMR #13831/M. Lochner
SB15b
D20 str

exp1 pulse sequence: s2pul

SAMPLE		DEC. & VT	
date	Mar 15 93	dn	H1
solvent	CDCl3	dof	0
file	/data/lin~	dm	nny
de/RR4/ab15b_31p		dmm	c
ACQUISITION		dmf	9284
sfrq	161.903	dhp	61
tn	P31	PROCESSING	
at	0.800	lb	2.00
np	64000	fn	65536
sw	40000.0	math	f
fb	22000		
bs	16	werr	
pw	7.3	wexp	
d1	1.000	wbs	
tof	12200.0	wnt	
nt	1e+06	DISPLAY	
ct	31552	sp	-0.0
alock	n	wp	8094.7
gain	25	vs	50
FLAGS		sc	0
il	n	wc	250
in	n	hzmm	73.08
dp	y	is	1.04281e+~ 06
hs	nn		
		rfl	7645.5
		rfp	0
		th	31
		ins	1.000
		nm cdc ph	



APPENDIX D

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR SAMPLE IDENTIFICATION

*1.1 NMR sample code:	1.2 Sample preparation date:
SN15-B	8 March 1993

SOLVENT

*1.3 Name and/or formula:	1.4 Deuteration degree or purity:	1.5 Manufacturer:
D_2O	99.9 atom % D	MSD Isotopes

CHEMICAL SHIFT REFERENCE COMPOUND(S)

*1.6 For nucleus	*1.7 Name and/or formula	*1.8 Type	1.9 Manufacturer	1.10 Purity
^{31}P	Phosphoric Acid	External	Fisher Sci. Co.	85%

1.11 Preparation of chemical shift reference solution(s):

Reference used as received.

NMR TUBE

1.12 Sealed by:	1.13 Outside diameter:	1.14 Tube manufacturer, and tube code:	1.15 Filtration method:
i) melting ii) a cap + Parafilm iii) other means, describe	5-mm	Wilmad 507-PP	i) no filtration ii) through piece of cotton wool iii) through a sintered glass iv) other, describe (see 1.16)

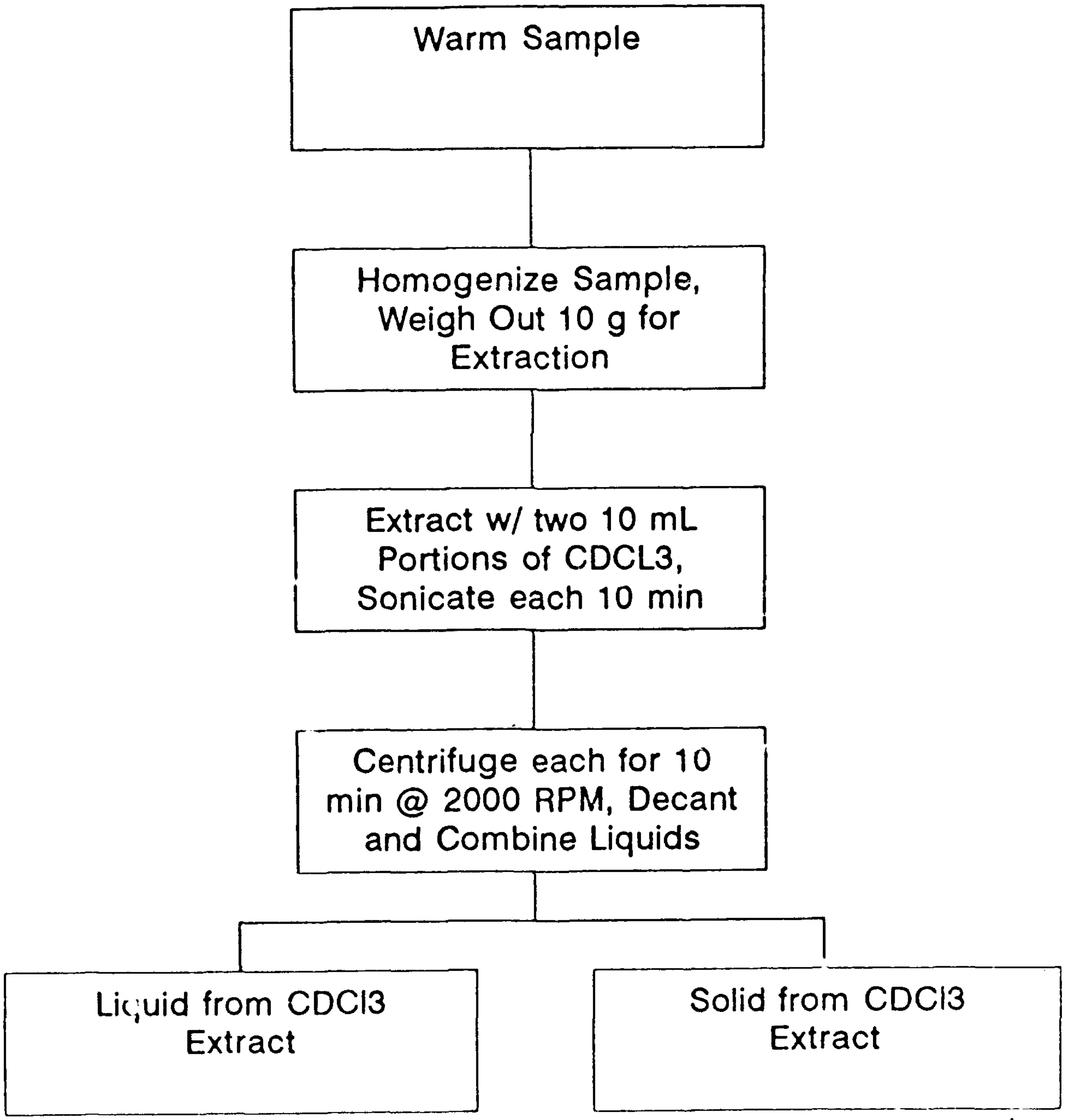
SAMPLE PREPARATION

*1.16 Sample preparation details:

See attached block diagram.

1.17 Notes

Soil Sample Preparation for NMR Analysis



Soil Sample Preparation for NMR Analysis (Continued)

Liquid from CDCl3 Extract

Dry w/ Anhydrous Sodium Sulfate

Filter thru 0.45 μm Disposable Filter

Concentrate to 1 mL w/ Dry Nitrogen

Transfer to NMR Tube for Analysis

Soil Sample Preparation for NMR Analysis (Continued)

Solid from CDCl3 Extract

Extract w/ two 10 mL Portions of D2O, Sonicate each 5 min

Centrifuge each for 10 min @ 2000 RPM, Filter and Combine Liquids

Solid

Add Two 10 mL portions of 0.5M CH3OH/KOH, Sonicate each 10 min

Centrifuge each 3 min @ 2000 RPM, Decant and Combine Liquids

Dry w/ Anhydrous Sodium Sulfate

Filter w/ 0.45 μm Disposable Filter

Concentrate to Dryness w/ Dry Nitrogen, Take-Up in 1.5 mL CD3OD

Transfer to NMR Tube for Analysis

Liquid

Concentrate to 1 mL w/ Dry Nitrogen

Transfer to NMR Tube for Analysis *

Sample SN-15B

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR INSTRUMENTATION			
*2.1 NMR spectrometer: Varian VXR-400S		2.2 Spectrometer manufacturer: Varian Associates	
2.3* Proton frequency [MHz]: 399.95 MHz	2.4 Temperature control unit present: YES / NO	2.5 Install. year: 1988	2.6 Spectrometer operating system: VNMRS 4.1B
*2.7 Probehead(s)			2.8 Computer operating system: SUNOS 4.1.2
*2.7.1 Probehead name	*2.7.2 Observable nuclei	*2.7.3 Sample diameter	2.9 Notes:
Broadband/switch-able	^1H, ^{19}F and ^{15}N thru ^{31}P	5-mm	

TESTING OF INSTRUMENT PERFORMANCE

*3.1 Nucleus	(3.2) *2.7.1 Probehead name	*3.3 Test date	*3.4 Test name, test sample, and sample origin	*3.5 Result (S/N, linewidth)	*3.6 Specification value
^1H	BB/swit.	2 Mar 93	Sensitivity, 0.1% ethyl benzene/$CDCl_3$ (Varian)	130	100
^{19}F	BB/swit.	2 Mar 93	Sensitivity, 0.05% CF_3-C_6H_5 in C_6D_6 (Varian)	215	100
^{13}C	BB/swit.	2 Mar 93	Sensitivity, 40% Dioxane, 60% C_6D_6 (Varian)	165	120
^{31}P	BB/swit.	2 Mar 93	Sensitivity, 0.0485M Tri-phenyl Phosphate/$CDCl_3$ (VA)	167	100
^{13}C	BB/swit.	16 Dec 92	Resolution, 40% Dioxane, 60% C_6D_6 (Varian)	0.15	≤ 0.2

3.7 Notes: S/N measurement used as criteria for instrument performance since S/N specification will not be met unless resolution and pulse width are within specifications.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*4.1 Method: 1H NMR; 13C-{1H} NMR; 19F NMR; 31P-{1H} NMR; 31P NMR; other, describe:			
*4.2 Spectrum number: NMR-8	(4.3) *1.1 NMR sample code: SN15-B	(4.4) *2.1 NMR spectrometer: Varian VXR-400S	4.5 Recording date: 8 March 1993
*4.6 Observation frequency [MHz]: 161.903	*4.7 Sample temperature [°C]: +22 °C Controlled: YES NO	*4.8 Spectral width [Hz]: 40,000	4.9 Spectral width [ppm]: 247 ppm
*4.10 Obs. pulse angle (degrees): 47°	*4.11 Obs. pulse duration (μs): 7.3	4.12 Pulse sequence name: S2PUL	*4.13 Number of scans: 35424
*4.14 Repetition time (s): 1.8	4.15 Total acquisition time: 18 hrs	*4.16 Number od data points in FID: 64,000	*4.17 Number of data points in real part of spectrum: 32,000
*4.18 Lock conditions: i) lock provided by solvent ii) experiment without lock iii) other lock system, describe	*4.19 $\nu_{(1/2)}$ and S/N: $\nu_{(1/2)}$= 50 Hz of line at 22.4 ppm S/N= 5/1 of line at 22.4 ppm	(4.20) *2.7.1 Probehead name: BB/Switchable	*4.21 Chemical shift reference value [ppm]: 0.00
4.22 Quantitation			
4.23 Notes:			

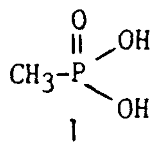
5. Analysis Results

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

Methylphosphonic Acid

*5.2 Molecular structure:
(with numbering of atoms)



*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
993-13-5	NMR-8	SN15-B

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{31}P NMR

5.8 Analysis date: 8 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	22.5	Not determined.

5.10 Notes:

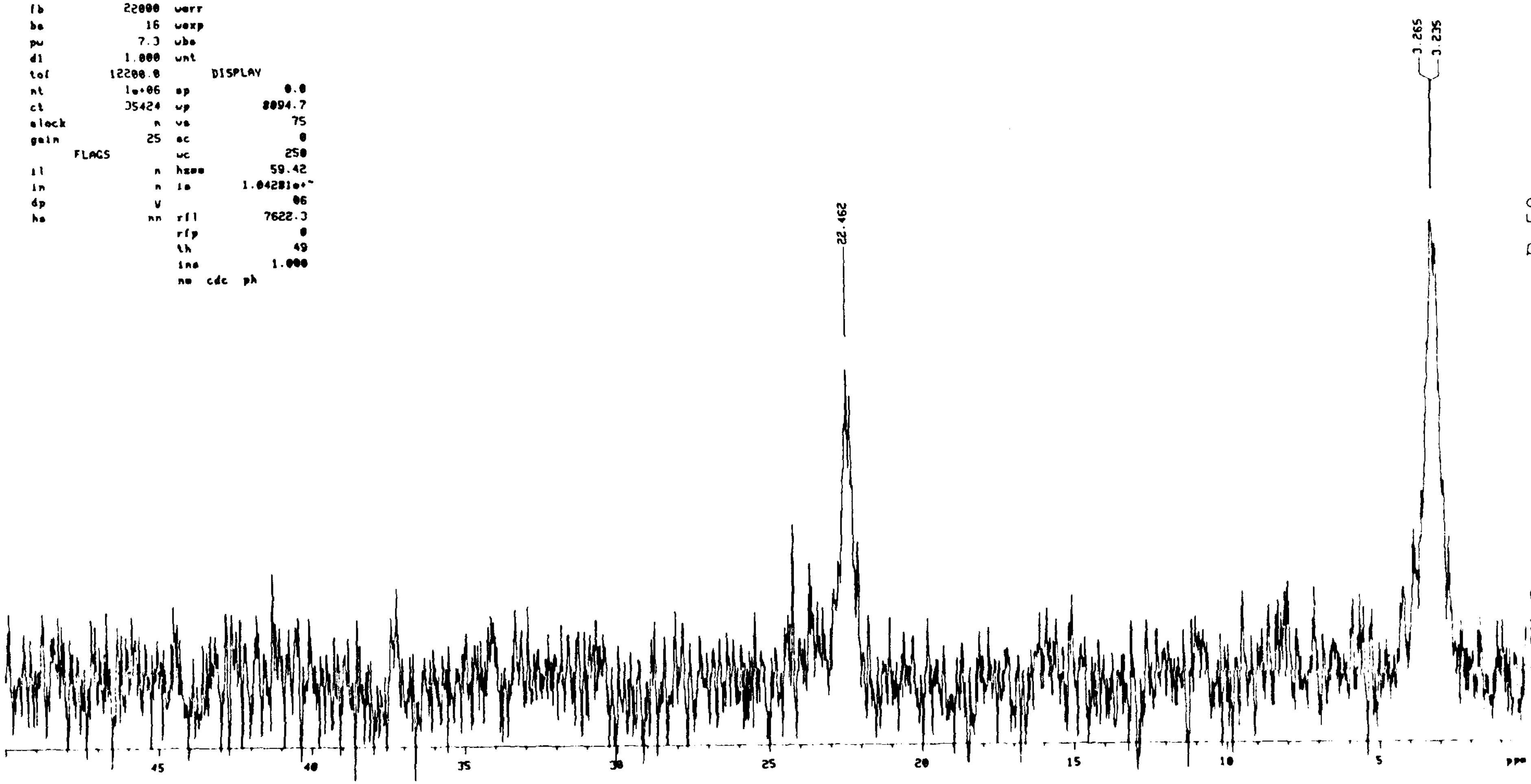
Resonance in phosphate region (delta 3.2 ppm) was observed in the blank sample (SNB15-B) also.

SPECTRUM NO: NMR-8

NMR #13827/M. Lochner
an15-B
D2O extract from
soil

exp1 pulse sequence: s2pul

SAMPLE		DEC. & VT	
date	Mar 8 93	dn	H1
solvent	CDCl3	dof	0
file	/data/lin~	dm	nny
da/RR4/an15_b_d2o_~		dmm	s
	31p	dmf	9284
ACQUISITION		dhp	61
sfrq	161.903	PROCESSING	
tn	P31	lb	5.00
at	0.800	fn	65536
np	64000	math	f
sw	40000.0		
fb	22000	werr	
bs	16	wexp	
pw	7.3	wbs	
d1	1.000	wnt	
tof	12200.0	DISPLAY	
nt	1e+06	sp	0.0
ct	35424	wp	8094.7
alock	n	vs	75
gain	25	sc	0
FLAGS		wc	250
il	n	hzmm	59.42
in	n	is	1.04281e+~
			06
dp	y		
hs	nn	rfl	7622.3
		rfp	0
		th	49
		ins	1.000
		nm cdc ph	



1. NMR Sample Preparation

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR SAMPLE IDENTIFICATION

*1.1 NMR sample code:	1.2 Sample preparation date:
SB15-C	9 March 1993

SOLVENT

*1.3 Name and/or formula:	1.4 Deuteration degree or purity:	1.5 Manufacturer:
CD_3OD	99.8 atom %D	Isotec, Inc.

CHEMICAL SHIFT REFERENCE COMPOUND(S)

*1.6 For nucleus	*1.7 Name and/or formula	*1.8 Type	1.9 Manufacturer	1.10 Purity
^1H	Tetramethylsilane (TMS)	Internal	Norell Chem. Co.	99.5%

1.11 Preparation of chemical shift reference solution(s):

Reference compound used as received.

NMR TUBE

1.12 Sealed by:	1.13 Outside diameter:	1.14 Tube manufacturer, and tube code:	1.15 Filtration method:
i) melting ii) a cap + Parafilm iii) other means, describe	5-mm	Wilmad 507-PP	i) no filtration ii) through piece of cotton wool iii) through a sintered glass iv) other, describe (see 1.16)

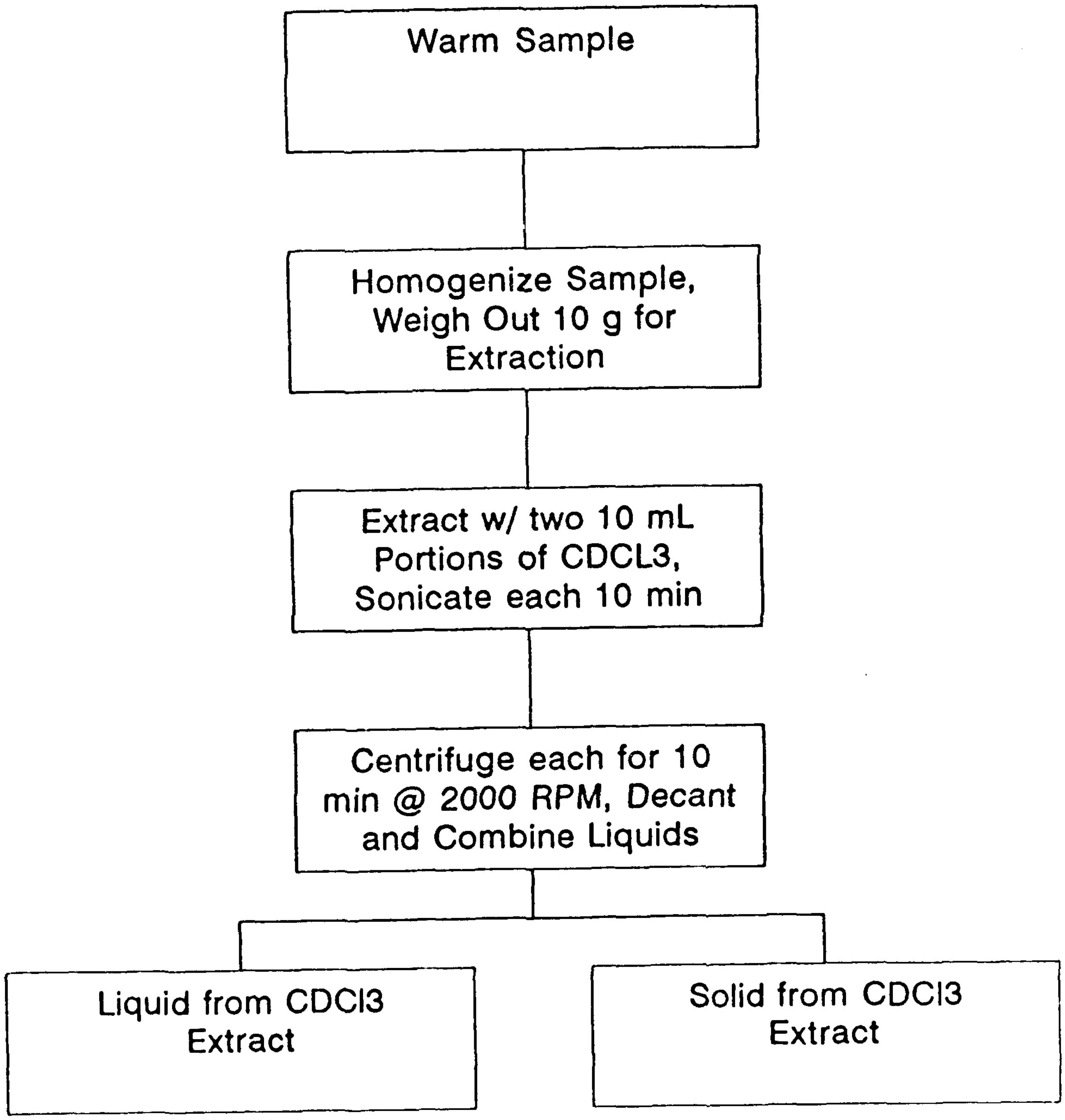
SAMPLE PREPARATION

*1.16 Sample preparation details:

See attached block diagram.

1.17 Notes

Soil Sample Preparation for NMR Analysis



Soil Sample Preparation for NMR Analysis (Continued)

Liquid from CDCl3 Extract

Dry w/ Anhydrous Sodium Sulfate

Filter thru 0.45 μm Disposable Filter

Concentrate to 1 mL w/ Dry Nitrogen

Transfer to NMR Tube for Analysis

Soil Sample Preparation for NMR Analysis (Continued)

Solid from CDCl3 Extract

Extract w/ two 10 mL Portions of D2O, Sonicate each 5 min

Centrifuge each for 10 min @ 2000 RPM, Filter and Combine Liquids

Solid

Add Two 10 mL portions of 0.5M CH3OH/KOH, Sonicate each 10 min

Centrifuge each 3 min @ 2000 RPM, Decant and Combine Liquids

Dry w/ Anhydrous Sodium Sulfate

Filter w/ 0.45 μm Disposable Filter

Concentrate to Dryness w/ Dry Nitrogen, Take-Up in 1.5 mL CD3OD

Transfer to NMR Tube for Analysis

*

Liquid

Concentrate to 1 mL w/ Dry Nitrogen

Transfer to NMR Tube for Analysis

Sample SB15-C

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR INSTRUMENTATION

*2.1 NMR spectrometer: Varian VXR-400S		2.2 Spectrometer manufacturer: Varian Associates	
2.3* Proton frequency [MHz]: 399.95 MHz	2.4 Temperature control unit present: YES / NO	2.5 install. year: 1988	2.6 Spectrometer operating system: VNMRS 4.1B
*2.7 Probehead(s)			2.8 Computer operating system: SUNOS 4.1.2
*2.7.1 Probehead name	*2.7.2 Observable nuclei	*2.7.3 Sample diameter	2.9 Notes:
Broadband/switch-able	^{1}H, ^{19}F and ^{15}N thru ^{31}P	5-mm	

TESTING OF INSTRUMENT PERFORMANCE

*3.1 Nucleus	(3.2) *2.7.1 Probehead name	*3.3 Test date	*3.4 Test name, test sample, and sample origin	*3.5 Result (S/N, linewidth)	*3.6 Specification value
^{1}H	BB/swit.	2 Mar 93	Sensitivity, 0.1% ethyl benzene/$CDCl_3$ (Varian)	130	100
^{19}F	BB/swit.	2 Mar 93	Sensitivity, 0.05% CF_3–C_6H_5 in C_6D_6 (Varian)	215	100
^{13}C	BB/swit.	2 Mar 93	Sensitivity, 40% Dioxane, 60% C_6D_6 (Varian)	165	120
^{31}P	BB/swit.	2 Mar 93	Sensitivity, 0.0485M Tri-phenyl Phosphate/$CDCl_3$ (VA)	167	100
^{13}C	BB/swit.	16 Dec 92	Resolution, 40% Dioxane, 60% C_6D_6 (Varian)	0.15	≤ 0.2

3.7 Notes: S/N measurement used as criteria for instrument performance since S/N specification will not be met unless resolution and pulse width are within specifications.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*4.1 Method: 1H NMR 13C-{1H} NMR 19F NMR 31P-{1H} NMR 31P NMR other, describe:

*4.2 Spectrum number: NMR-9	(4.3) *1.1 NMR sample code: SB15-C	(4.4) *2.1 NMR spectrometer: Varian VXR-400S	4.5 Recording date: 9 March 1993
*4.6 Observation frequency [MHz]: 399.952	*4.7 Sample temperature [°C]: +22 °C Controlled: YES NO	*4.8 Spectral width [Hz]: 8000	4.9 Spectral width [ppm]: 20 ppm
*4.10 Obs. pulse angle (degrees): 12°	*4.11 Obs. pulse duration (µs): 4.0	4.12 Pulse sequence name: S2PUL	*4.13 Number of scans: 320
*4.14 Repetition time (s): 5.0	4.15 Total acquisition time: 27 min	*4.16 Number od data points in FID: 32,000	*4.17 Number of data points in real part of spectrum: 16,000
*4.18 Lock conditions: i) lock provided by solvent ii) experiment without lock iii) other lock system, describe	*4.19 $\upsilon_{(1/2)}$ and S/N: $\upsilon_{(1/2)}$= 1.1 Hz of line at 6.32 ppm S/N= 3.6 of line at 7.32 ppm	(4.20) *2.7.1 Probehead name: BB/Switchable	*4.21 Chemical shift reference value [ppm]: 0.00

4.22 Quantitation

4.23 Notes:

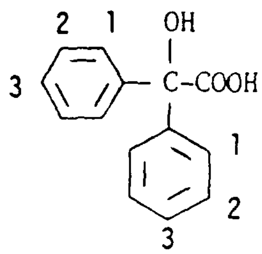
5. Analysis Results

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

Benzilic Acid

*5.2 Molecular structure: (with numbering of atoms)



*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
76-93-7	NMR-9	SB15-C

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^1H NMR

5.8 Analysis date: 9 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	7.53	"Doublet"; 2 protons.
2	7.32	"Triplet"; 2 protons.
3	7.26	"Triplet"; 1 proton.

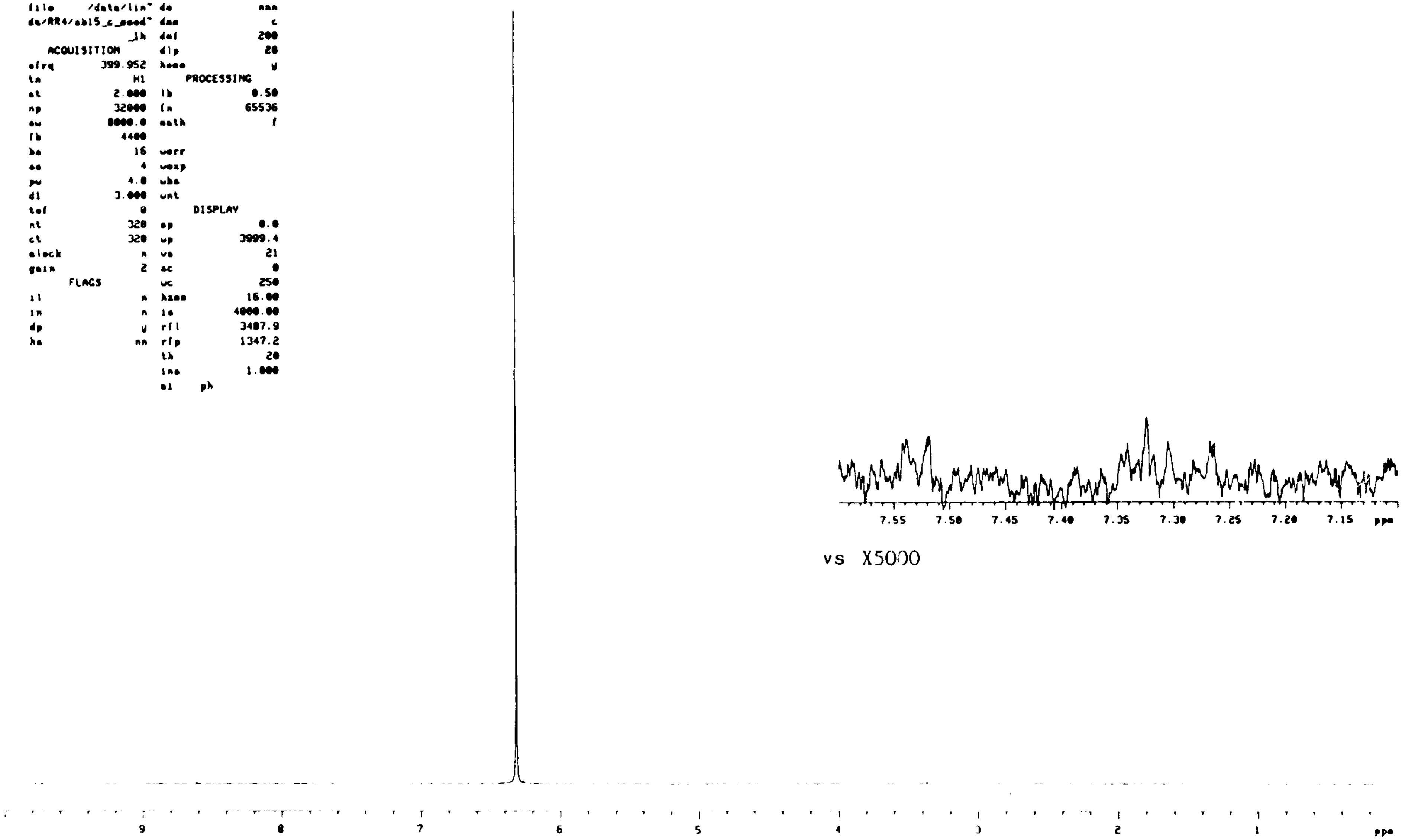
5.10 Notes: Spectrum not first order; no coupling constants determined.

NMR 8:3837/M. Lochner
SB15 C)
CD3OD/KOH extract
from soil

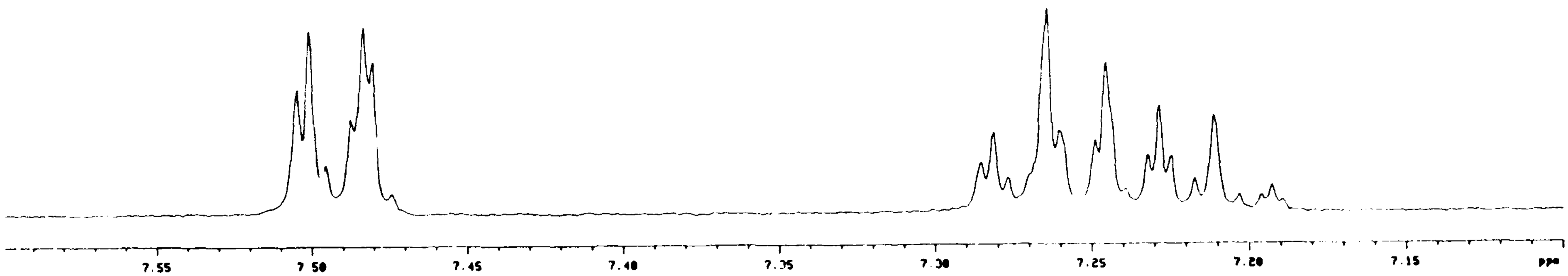
exp2 pulse sequence: stdlh

SAMPLE		DEC. & VT	
date	Mar 9 93	dn	H1
solvent	CD3OD	dof	0
file	/data/lin~	dm	nnn
da/RR4/sb15_c_mood~		dmm	c
	_1h	dmf	200
ACQUISITION		dlp	20
sfrq	399.952	homo	y
tn	H1	PROCESSING	
at	2.000	lb	0.50
np	32000	fn	65536
sw	8000.0	math	f
fb	4400		
bs	16	werr	
ss	4	wexp	
pw	4.0	wbs	
d1	3.000	wnt	
tof	0	DISPLAY	
nt	320	sp	0.0
ct	320	wp	3999.4
alock	n	vs	21
gain	2	sc	0
FLAGS		wc	250
il	n	hzmm	16.00
in	n	is	4000.00
dp	y	rfl	3487.9
hs	nn	rfp	1347.2
		th	20
		ins	1.000
		ai	ph

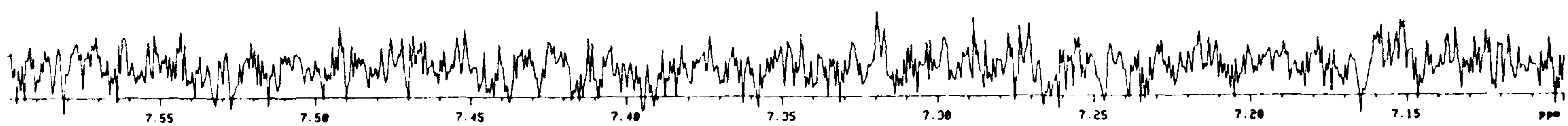
SPECTRUM NO: NMR-9



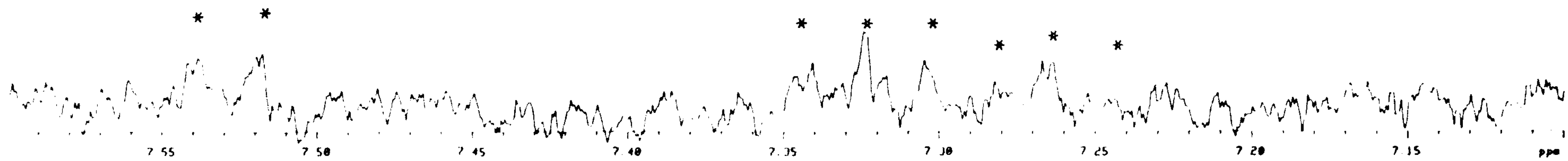
SPECTRUM NO: NMR-9



SPECTRUM OF AUTHENTIC COMPOUND.



SPECTRUM OF BLANK (SBB15-C).



SPECTRUM OF SAMPLE SB15-C.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR SAMPLE IDENTIFICATION

*1.1 NMR sample code:	1.2 Sample preparation date:
SN15-C	9 March 1993

SOLVENT

*1.3 Name and/or formula:	1.4 Deuteration degree or purity:	1.5 Manufacturer:
CD_3OD	99.8 atom %D	Isotec, Inc.

CHEMICAL SHIFT REFERENCE COMPOUND(S)

*1.6 For nucleus	*1.7 Name and/or formula	*1.8 Type	1.9 Manufacturer	1.10 Purity
^1H	Tetramethylsilane (TMS)	Internal	Norell Chem. Co.	99.5%

1.11 Preparation of chemical shift reference solution(s):

Reference compound used as received.

NMR TUBE

1.12 Sealed by:	1.13 Outside diameter:	1.14 Tube manufacturer, and tube code:	1.15 Filtration method:
i) melting ii) a cap + Parafilm iii) other means, describe	5-mm	Wilmad 507-PP	i) no filtration ii) through piece of cotton wool iii) through a sintered glass iv) other, describe (see 1.16)

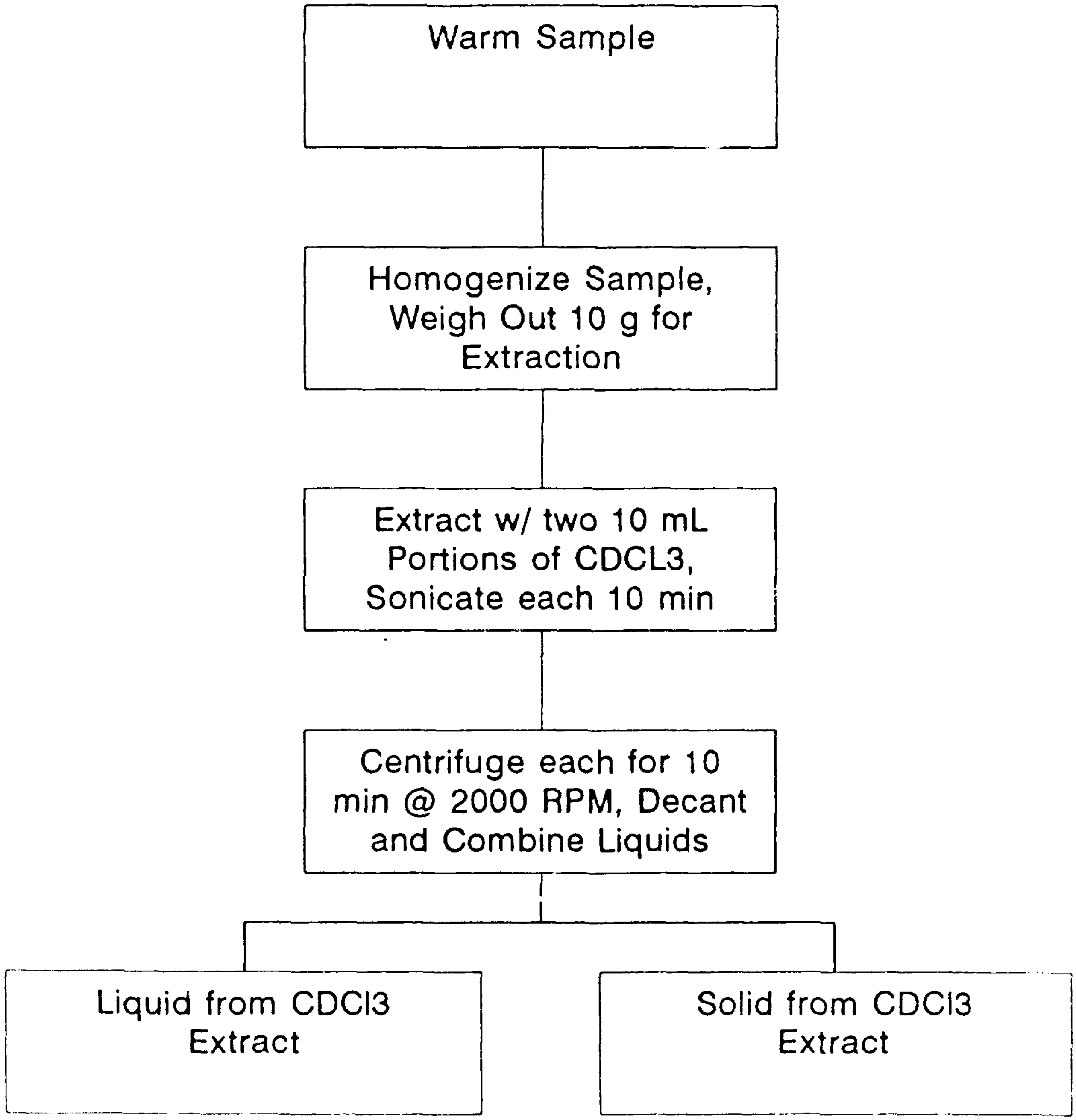
SAMPLE PREPARATION

*1.16 Sample preparation details:

See attached block diagram.

1.17 Notes

Soil Sample Preparation for NMR Analysis



Soil Sample Preparation for NMR Analysis (Continued)

Liquid from CDCl3 Extract

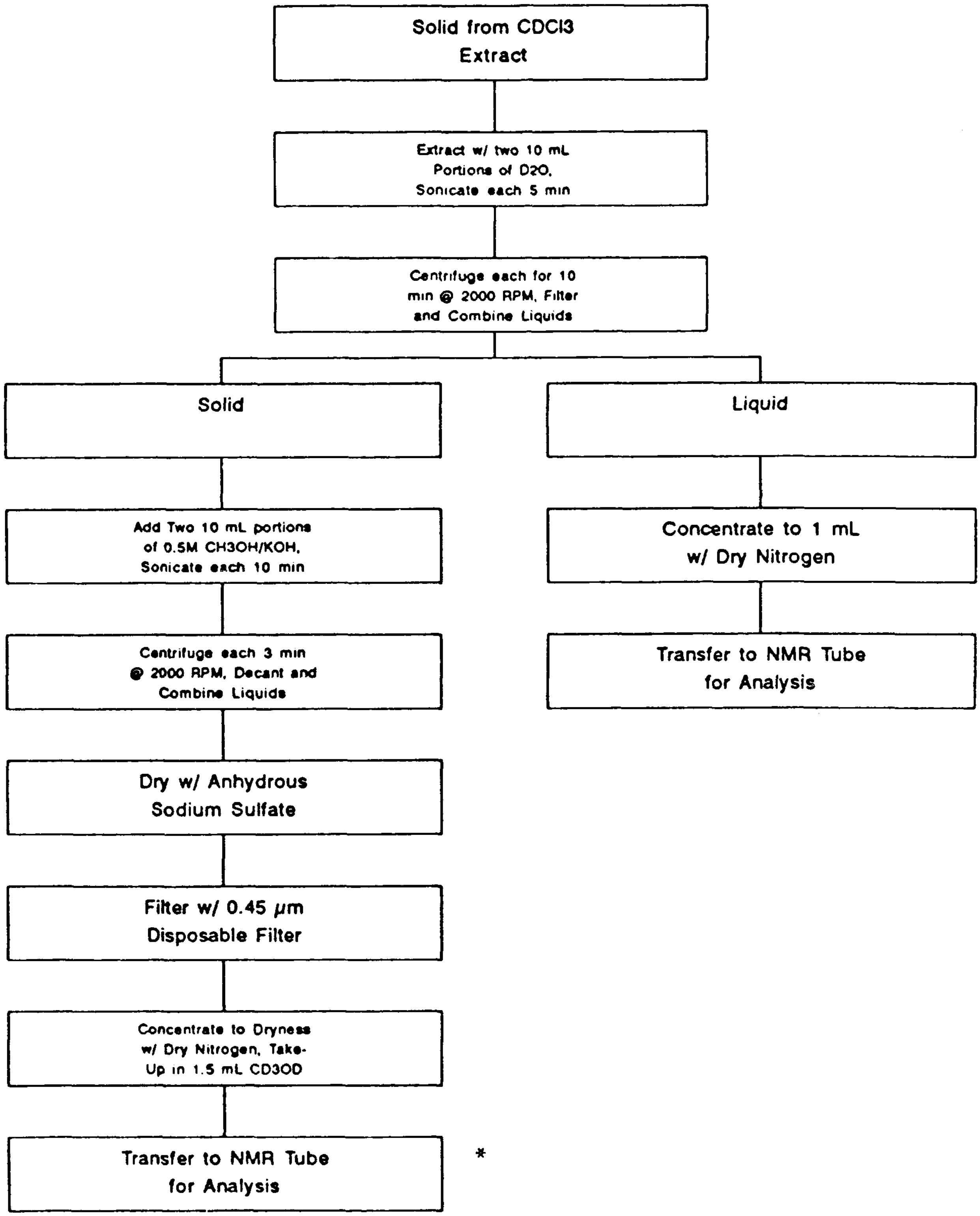
Dry w/ Anhydrous Sodium Sulfate

Filter thru 0.45 μm Disposable Filter

Concentrate to 1 mL w/ Dry Nitrogen

Transfer to NMR Tube for Analysis

Soil Sample Preparation for NMR Analysis (Continued)



Sample SN15-C

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR INSTRUMENTATION

*2.1 NMR spectrometer: Varian VXR-400S		2.2 Spectrometer manufacturer: Varian Associates	
2.3* Proton frequency [MHz]: 399.95 MHz	2.4 Temperature control unit present: YES (circled) / NO	2.5 Install. year: 1988	2.6 Spectrometer operating system: VNMRS 4.1B
*2.7 Probehead(s)			2.8 Computer operating system: SUNOS 4.1.2
*2.7.1 Probehead name	*2.7.2 Observable nuclei	*2.7.3 Sample diameter	2.9 Notes:
Broadband/switch-able	^{1}H, ^{19}F and ^{15}N thru ^{31}P	5-mm	

TESTING OF INSTRUMENT PERFORMANCE

*3.1 Nucleus	(3.2) *2.7.1 Probehead name	*3.3 Test date	*3.4 Test name, test sample, and sample origin	*3.5 Result (S/N, linewidth)	*3.6 Specification value
^{1}H	BB/swit.	2 Mar 93	Sensitivity, 0.1% ethyl benzene/$CDCl_3$ (Varian)	130	100
^{19}F	BB/swit.	2 Mar 93	Sensitivity, 0.05% CF_3-C_6H_5 in C_6D_6 (Varian)	215	100
^{13}C	BB/swit.	2 Mar 93	Sensitivity, 40% Dioxane, 60% C_6D_6 (Varian)	165	120
^{31}P	BB/swit.	2 Mar 93	Sensitivity, 0.0485M Tri-phenyl Phosphate/$CDCl_3$ (VA)	167	100
^{13}C	BB/swit.	16 Dec 92	Resolution, 40% Dioxane, 60% C_6D_6 (Varian)	0.15	$\leq$0.2

3.7 Notes: S/N measurement used as criteria for instrument performance since S/N specification will not be met unless resolution and pulse width are within specifications.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

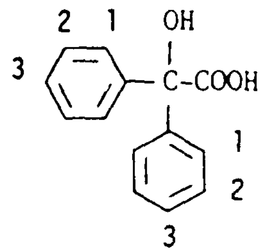
*4.1 Method: 1H NMR 13C-{1H} NMR 19F NMR 31P-{1H} NMR 31P NMR other, describe:			
*4.2 Spectrum number: NMR-10	(4.3) *1.1 NMR sample code: SN15-C	(4.4) *2.1 NMR spectrometer: Varian VXR-400S	4.5 Recording date: 11 March 1993
*4.6 Observation frequency [MHz]: 399.952	*4.7 Sample temperature [°C]: + 22 °C Controlled: YES NO	*4.8 Spectral width [Hz]: 8000	4.9 Spectral width [ppm]: 20 ppm
*4.10 Obs. pulse angle (degrees): 12°	*4.11 Obs. pulse duration (μs): 4.0	4.12 Pulse sequence name: S2PUL	*4.13 Number of scans: 816
*4.14 Repetition time (s): 4.0	4.15 Total acquisition time: 55 min	*4.16 Number od data points in FID: 48,000	*4.17 Number of data points in real part of spectrum: 24,000
*4.18 Lock conditions: i) lock provided by solvent ii) experiment without lock iii) other lock system, describe	*4.19 $\nu_{(1/2)}$ and S/N: $\nu_{(1/2)}$= 1.1 Hz of line at 6.45 ppm S/N= 6.4 of line at 7.32 ppm	(4.20) *2.7.1 Probehead name: BB/Switchable	*4.21 Chemical shift reference value [ppm]: 0.00
4.22 Quantitation			
4.23 Notes:			

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

Benzilic Acid

*5.2 Molecular structure:
(with numbering of atoms)



*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
76-93-7	NMR- 10	SN15-C

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{1}H NMR

5.8 Analysis date: 11 March 1993

*5.9 Interpretation

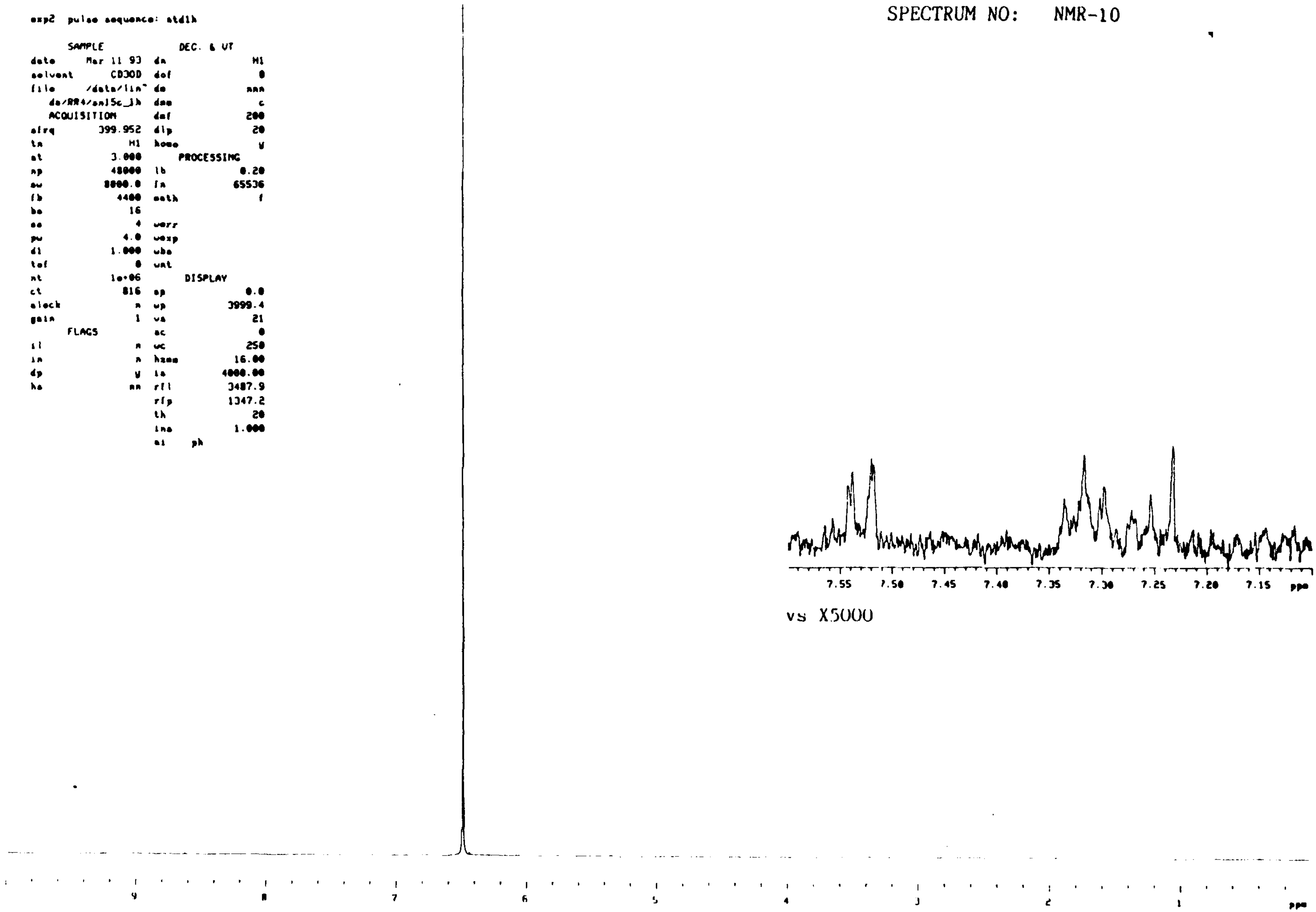
5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	7.53	"Doublet"; 2 protons.
2	7.32	"Triplet"; 2 protons.
3	7.25	"Triplet"; 1 proton.

5.10 Notes:

Spectrum not first order; no coupling constants determined.

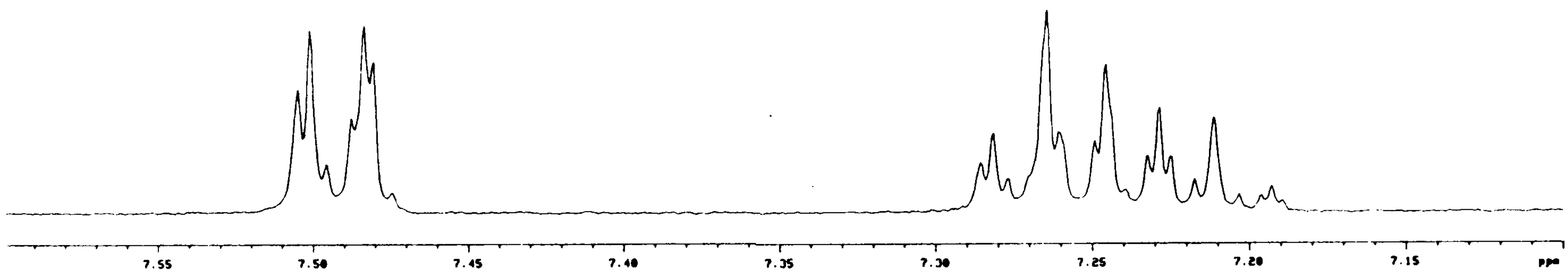
SPECTRUM NO: NMR-10

```
NMR 013832/M. Lechner
SM15c
MeOD/KOH str

exp2  pulse sequence: stdlh

      SAMPLE              DEC. & VT
date      Mar 11 93  dn              H1
solvent       CD3OD  dof              0
file     /data/lin"  dm             nnn
   da/RR4/sn15c_1h   dmm              c
   ACQUISITION       dmf            200
sfrq        399.952  dlp             20
tn               H1  homo             y
at            3.000       PROCESSING
np            48000  lb            0.20
sw           8000.0  fn           65536
fb             4400  math             f
bs               16
ss                4  werr
pw              4.0  wexp
d1            1.000  wbs
tof               0  wnt
nt            1e+06        DISPLAY
ct              816  sp             0.0
alock             n  wp          3999.4
gain              1  vs              21
      FLAGS          sc               0
il                n  wc             250
in                n  hzmm         16.00
dp                y  is         4000.00
hs               nn  rfl         3487.9
                     rfp         1347.2
                     th              20
                     ins          1.000
                     ai    ph
```

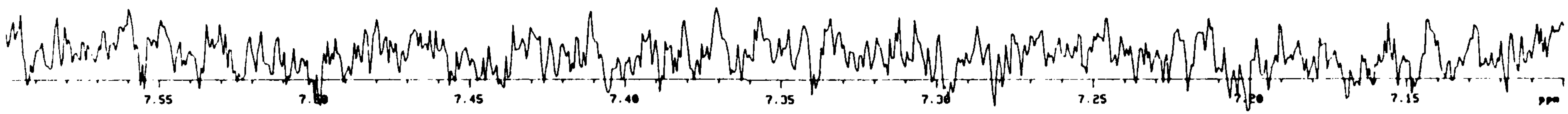


APPENDIX D

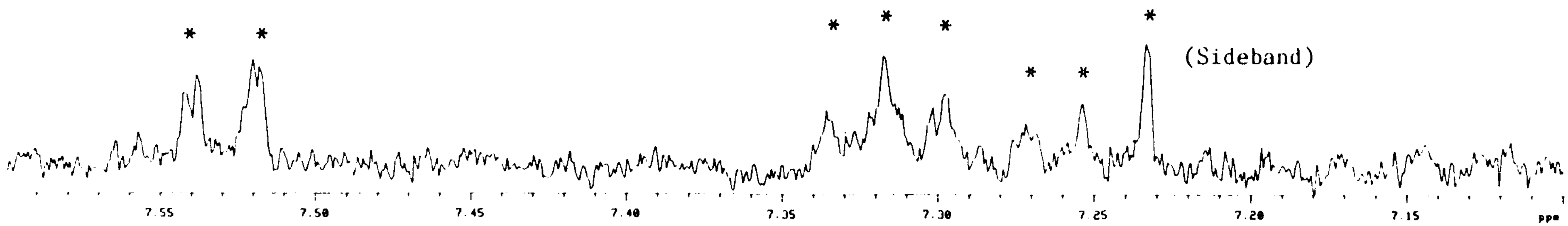
SPECTRUM NO: NMR-10



SPECTRUM OF AUTHENTIC COMPOUND.



SPECTRUM OF BLANK (SNB15-C).



SPECTRUM OF SAMPLE SN15-C.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR SAMPLE IDENTIFICATION

*1.1 NMR sample code: SA15-B	1.2 Sample preparation date: 8 March 1993

SOLVENT

*1.3 Name and/or formula: D_2O	1.4 Deuteration degree or purity: 99.9 atom % D	1.5 Manufacturer: MSD Isotopes

CHEMICAL SHIFT REFERENCE COMPOUND(S)

*1.6 For nucleus	*1.7 Name and/or formula	*1.8 Type	1.9 Manufacturer	1.10 Purity
^{31}P	Phosphoric Acid	External	Fisher Sci. Co.	85%

1.11 Preparation of chemical shift reference solution(s):

Reference used as received.

NMR TUBE

1.12 Sealed by:	1.13 Outside diameter:	1.14 Tube manufacturer, and tube code:	1.15 Filtration method:
i) melting ii) a cap + Parafilm iii) other means, describe	5-mm	Wilmad 507-PP	i) no filtration ii) through piece of cotton wool iii) through a sintered glass iv) other, describe (see 1.16)

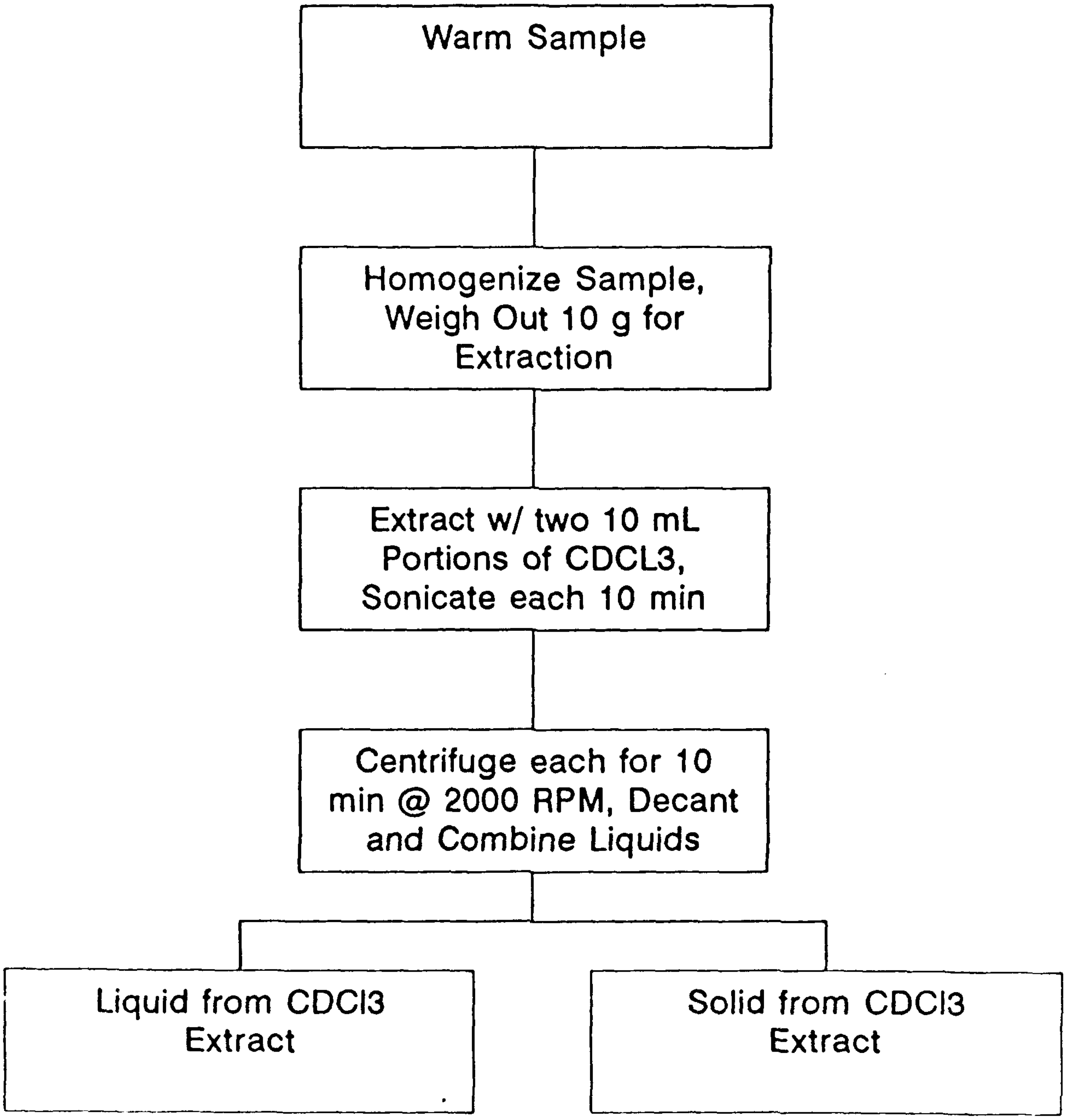
SAMPLE PREPARATION

*1.16 Sample preparation details:

See attached block diagram.

1.17 Notes

Soil Sample Preparation for NMR Analysis



Soil Sample Preparation for NMR Analysis (Continued)

Liquid from CDCl3 Extract

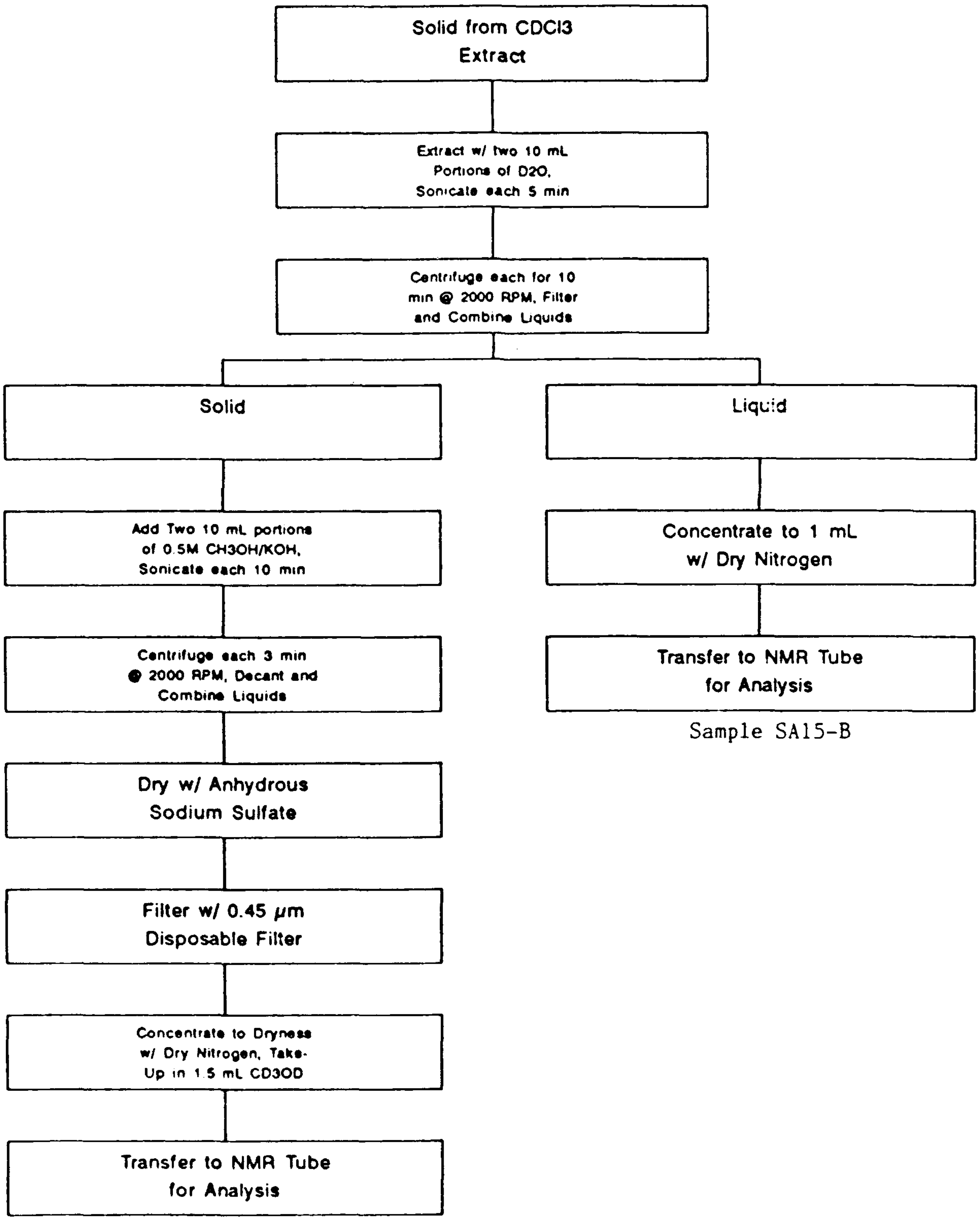
Dry w/ Anhydrous Sodium Sulfate

Filter thru 0.45 μm Disposable Filter

Concentrate to 1 mL w/ Dry Nitrogen

Transfer to NMR Tube for Analysis

Soil Sample Preparation for NMR Analysis (Continued)



*

Sample SA15-B

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

NMR INSTRUMENTATION

*2.1 NMR spectrometer: Varian VXR-400S		2.2 Spectrometer manufacturer: Varian Associates	
2.3* Proton frequency [MHz]: 399.95 MHz	2.4 Temperature control unit present: YES / NO (YES circled)	2.5 Install. year: 1988	2.6 Spectrometer operating system: VNMRS 4.1B
*2.7 Probehead(s)			2.8 Computer operating system: SUNOS 4.1.2
*2.7.1 Probehead name	*2.7.2 Observable nuclei	*2.7.3 Sample diameter	2.9 Notes:
Broadband/switch-able	^1H, ^{19}F and ^{15}N thru ^{31}P	5-mm	

TESTING OF INSTRUMENT PERFORMANCE

*3.1 Nucleus	(3.2) *2.7.1 Probehead name	*3.3 Test date	*3.4 Test name, test sample, and sample origin	*3.5 Result (S/N, linewidth)	*3.6 Specification value
^1H	BB/swit.	2 Mar 93	Sensitivity, 0.1% ethyl benzene/$CDCl_3$ (Varian)	130	100
^{19}F	BB/swit.	2 Mar 93	Sensitivity, 0.05% CF_3-C_6H_5 in C_6D_6 (Varian)	215	100
^{13}C	BB/swit.	2 Mar 93	Sensitivity, 40% Dioxane, 60% C_6D_6 (Varian)	165	120
^{31}P	BB/swit.	2 Mar 93	Sensitivity, 0.0485M Tri-phenyl Phosphate/$CDCl_3$ (VA)	167	100
^{13}C	BB/swit.	16 Dec 92	Resolution, 40% Dioxane, 60% C_6D_6 (Varian)	0.15	≤ 0.2

3.7 Notes: S/N measurement used as criteria for instrument performance since S/N specification will not be met unless resolution and pulse width are within specifications.

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*4.1 Method: 1H NMR   13C-{1H} NMR   19F NMR
31P-{1H} NMR   31P NMR   other, describe:

*4.2 Spectrum number: NMR-11	(4.3) *1.1 NMR sample code: SA15-B	(4.4) *2.1 NMR spectrometer: Varian VXR-400S	4.5 Recording date: 19 March 1993
*4.6 Observation frequency [MHz]: 161.903	*4.7 Sample temperature [°C]: +22 °C Controlled: YES / NO	*4.8 Spectral width [Hz]: 40,000	4.9 Spectral width [ppm]: 247 ppm
*4.10 Obs. pulse angle (degrees): 47°	*4.11 Obs. pulse duration (µs): 7.3	4.12 Pulse sequence name: S2PUL	*4.13 Number of scans: 126,544
*4.14 Repetition time (s): 1.8	4.15 Total acquisition time: 63.5 hrs	*4.16 Number od data points in FID: 64,000	*4.17 Number of data points in real part of spectrum: 32,000
*4.18 Lock conditions: i) lock provided by solvent ii) experiment without lock iii) other lock system, describe	*4.19 $\nu_{(1/2)}$ and S/N: $\nu_{(1/2)}$= 550 Hz of line at 24.9 ppm S/N= 3.4/1 of line at 24.9 ppm	(4.20) *2.7.1 Probehead name: BB/Switchable	*4.21 Chemical shift reference value [ppm]: 0.00

4.22 Quantitation

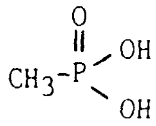
4.23 Notes:

Laboratory/Country: LABORATORY 15 / U.S.A. (ABERDEEN)

*5.1 IUPAC, CA, or trivial name of the identified compound:

Methylphosphonic Acid

*5.2 Molecular structure:
(with numbering of atoms)



1

*5.3 CAS No:	(5.4) *4.2 Spectrum number:	(5.5) *1.1 NMR sample code:
993-13-5	NMR- 11	SA15-B

*5.6 Criteria for positive identification:
i) interpretation
ii) comparison to a database spectrum
iii) comparison to a reference spectrum of authentic compound

(5.7) *4.1 Method: ^{31}P NMR

5.8 Analysis date: 19 March 1993

*5.9 Interpretation

5.9.1 Assignment (see 5.2 for numbering of atoms)	5.9.2 Chemical shifts [ppm]	5.9.3 Coupling constants [Hz]
1	24.9	Not determined.

5.10 Notes: The resonance in the phosphate region (delta 1.1 ppm) was observed in the blank sample (SAB15-B) also.

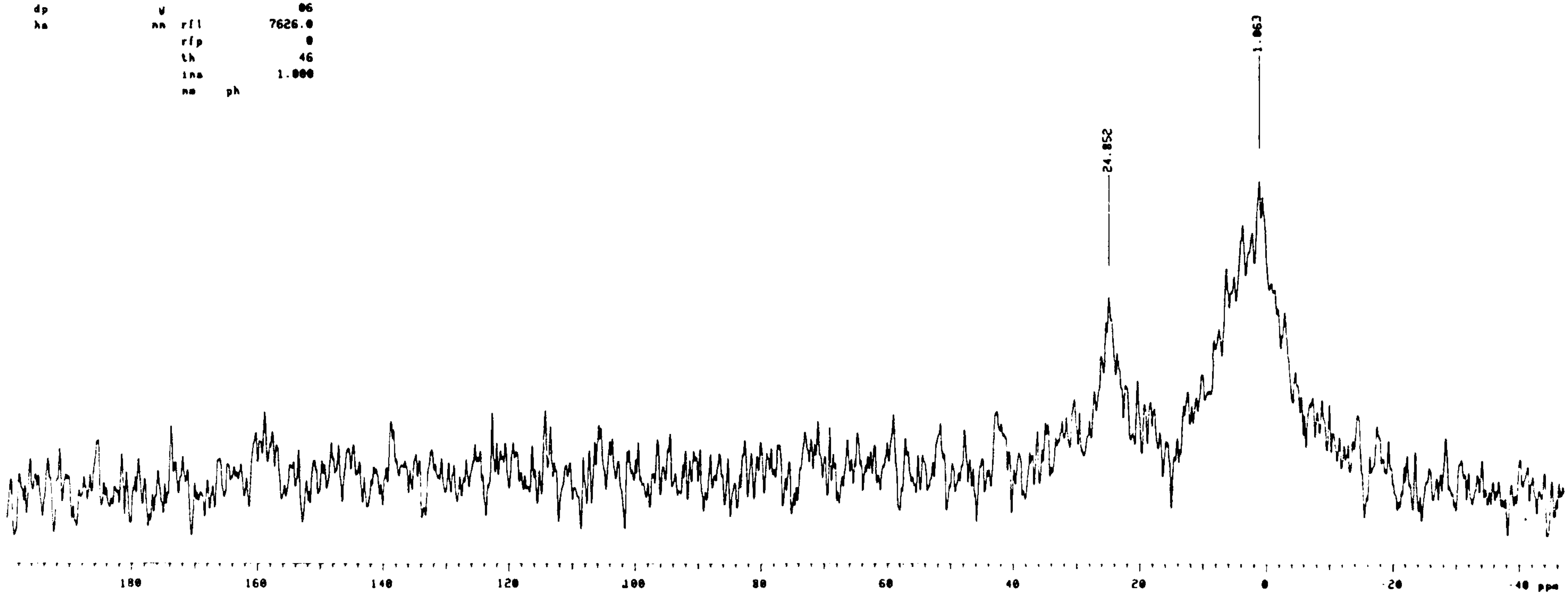
Since all samples are spiked with the same compounds, the presence of methylphosphonic acid in this sample is confirmed by the resonance observed in the C-P region of the spectrum.

SPECTRUM NO: NMR-11

NMR 813830/M. Lochner
SA15 (B)
D2O extract
from Soil

exp1 pulse sequence: s2pul

SAMPLE		DEC. & VT	
date	Mar 19 93	dn	H1
solvent	CDCl3	dof	0
file	/data/lin~	dm	nny
da/RR4/sa15b_31p_1~		dmm	s
	ong	dmf	9284
ACQUISITION		dhp	61
sfrq	161.983	PROCESSING	
tn	P31	lb	50.00
at	0.800	fn	65536
np	64000	math	f
sw	40000.0		
fb	22000	werr	
bs	16	wexp	
pw	7.3	wbs	
d1	1.000	wnt	
tof	12200.0	DISPLAY	
nt	1e+06	sp	-7626.0
ct	126544	wp	40000.0
alock	n	vs	51
gain	25	sc	0
FLAGS		wc	250
il	n	hzmm	73.89
in	n	is	1.04281e+~ 06
dp	y		
hs	nn	rfl	7626.0
		rfp	0
		th	46
		ins	1.000
		nm	ph



Blank

APPENDIX E

GC/MS METHODS AND MASS SPECTRA

Table E-1. GC/MS Operating Parameters

MS INSTRUMENTATION AND OPERATING CONDITIONS

Instrument; MS Type: HP-MSD 5750 SQ (MS1)
Finnigan ITD 40 Ion Trap (MS2, MS4)
Finnigan 5100 SQ (MS3)

Operating Parameters:	**Operating Conditions in MS modes:** MS1 EILRMS	MS2 EILRMS	MS3 CILRMS	MS4 CILRMS
GC Parameters (see App F)	GC1	GC2	GC3, GC7	GC2
Source temperature (°C)	200	220	120	220
CI source pressure (Torr)			0.5	12
Electron energy (eV)	70	AGC[1]	70	ARC[2]
Emission current (mA)	0.3	0.1	0.5	0.1
Scan range (m/z)	35-450	38-650	60-450	80-400
Scan time (s)	0.6	1.0	1.0	1.0
Reaction Gas			Methane	Methane

[1] Automatic Gain Control [2] Automatic Reaction Control

Table E-2. Scheduled Compounds Detected By GC/MS

Identified Compounds Peak Number and Name	Identification Criteria (Derivatives/References) EI	CI (CH_4)
WATER SAMPLE W-15		
1 Methylphosphonic Acid	C/D	C/E
2 2-Diisopropylaminoethanol	A/D, C/D	A/E, C/D
3 3-Quinuclidinol	C/D	C/D
4 Benzilic Acid	B/D, C/D	B/D, C/D

Table E-2. Scheduled Compounds Detected By GC/MS

Identified Compounds Peak Number and Name	Identification Criteria (Derivatives/References) EI	CI (CH_4)
WATER SAMPLE KEW-14		
1 Methylphosphonic Acid	-	C/E
2 2-Diisopropylaminoethanol	A/D	A/D, C/E
3 3-Quinuclidinol	C/D	C/D
4 Benzilic Acid	B/D, C/D	B/D, C/E
CARTRIDGE SAMPLE K-15		
2 2-Diisopropylaminoethanol	-	A/D
3 3-Quinuclidinol	-	C/E
4 Benzilic Acid	-	B/D
SOIL SAMPLE SA-15		
1 Methylphosphonic Acid	-	C/E
2 2-Diisopropylaminoethanol	C/D	C/E
3 3 Quinuclidinol	-	C/E
4 Benzilic Acid	C/D	C/E
SOIL SAMPLE SB-15		
1 Methylphosphonic Acid	-	B/E
2 2-Diisopropylaminoethanol	-	A/E, C/E
3 3-Quinuclidinol	C/D	C/D
4 Benzilic Acid	B/D, C/D	B/D, C/D
SOIL SAMPLE SN-15		
1 Methylphosphonic Acid	B/D, C/D	B/D, C/D
2 2-Diisopropylaminoethanol	A/D	A/D, C/E
3 3-Quinuclidinol	C/D	C/D
4 Benzilic Acid	B/D, C/D	B/D

Derivative Code:
A Identified as Original Compound
B Identified as Methyl Ester
C Identified as TMS Derivative

Reference Code:
D Full Scan Compared With Authentic Reference Compound
E SIM Only Compared With Authentic Reference Compound

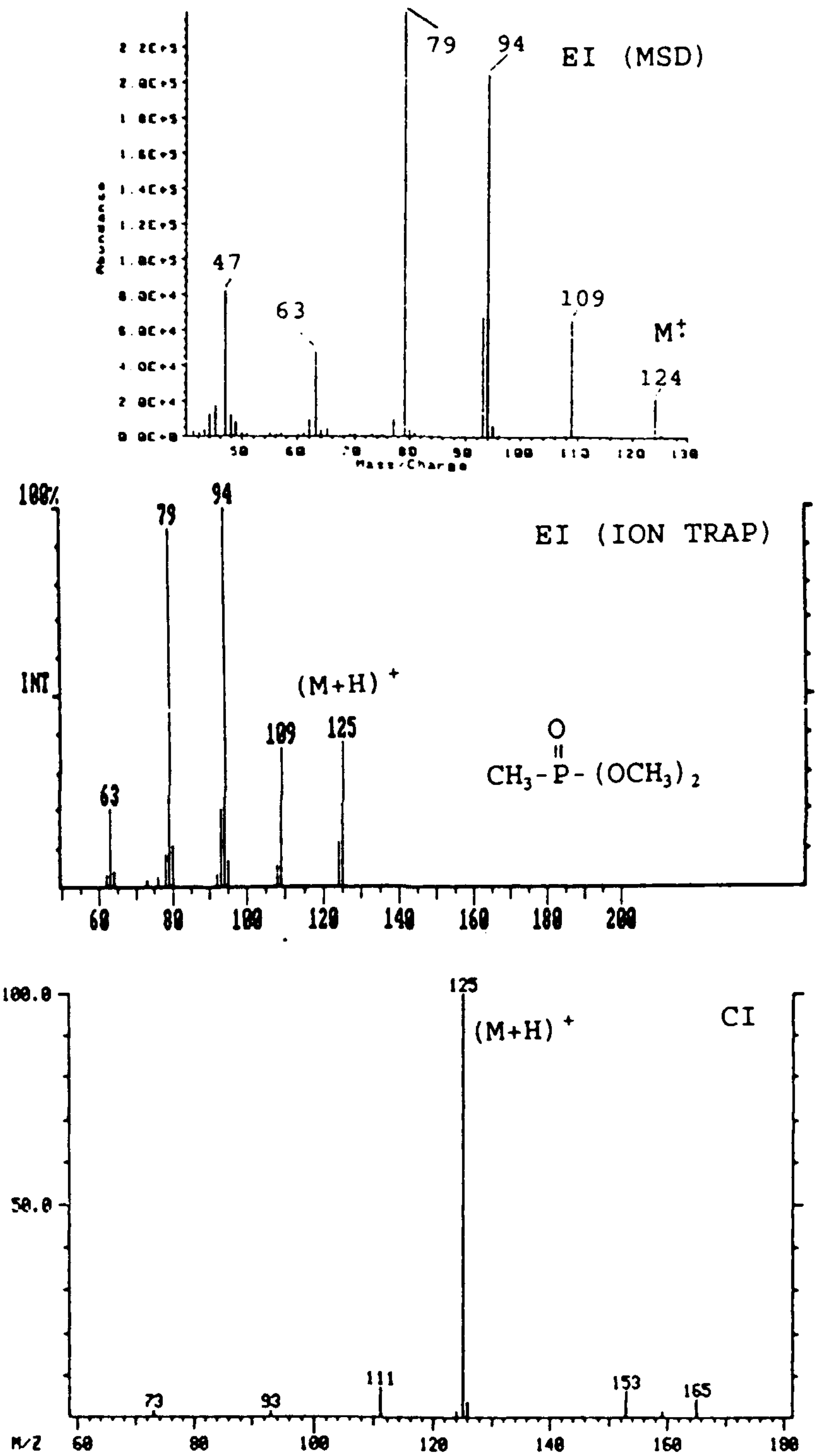


Figure E-1. EI (MSD, Ion Trap) and Methane CI Mass Spectra of Methylphosphonic Acid, Dimethyl Derivative

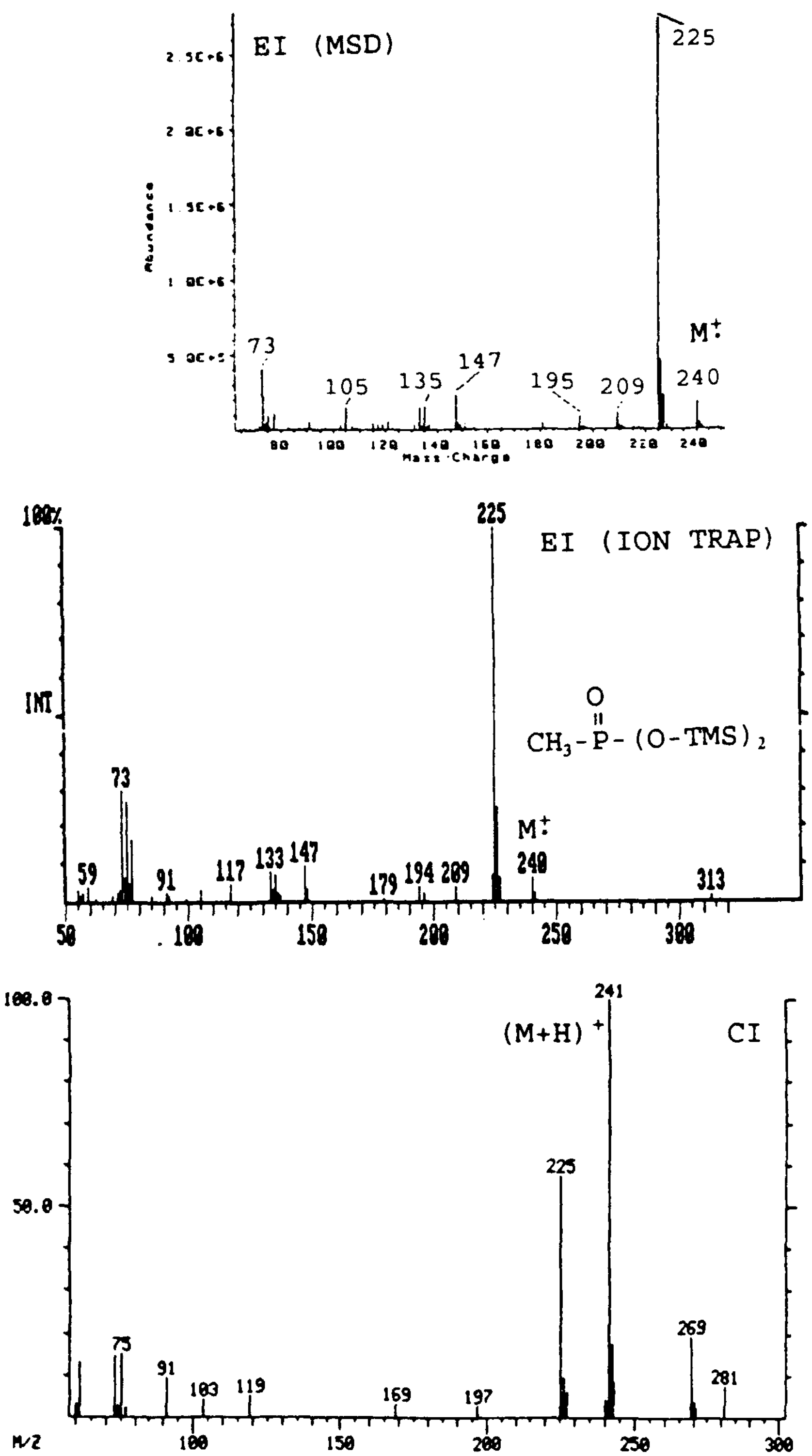


Figure E-2. EI (MSD, Ion Trap) and Methane CI Mass Spectra of Methylphosphonic Acid, Di-TMS Derivative

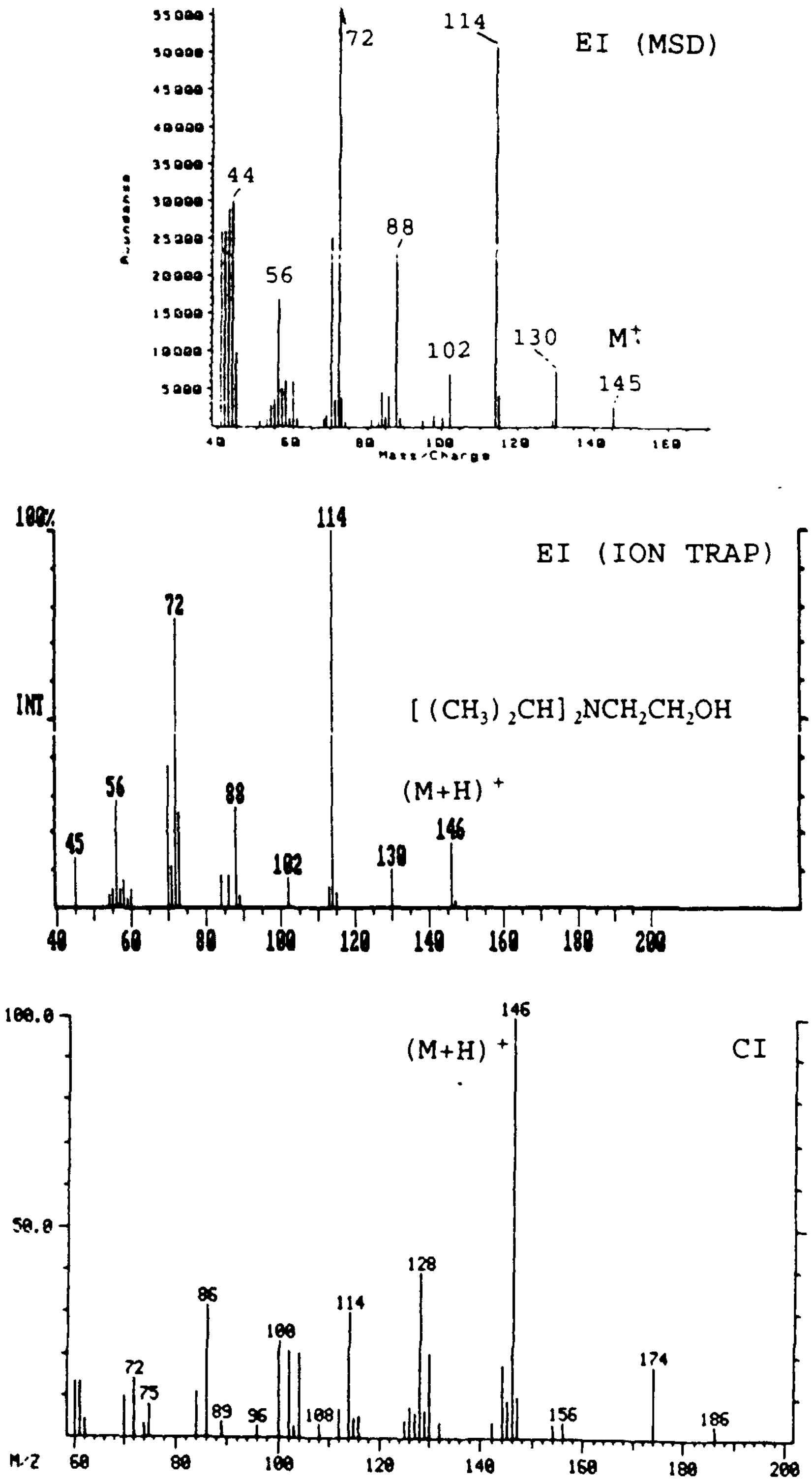


Figure E-3. EI (MSD, Ion Trap) and Methane CI Mass Spectra of 2-Diisopropylaminoethanol

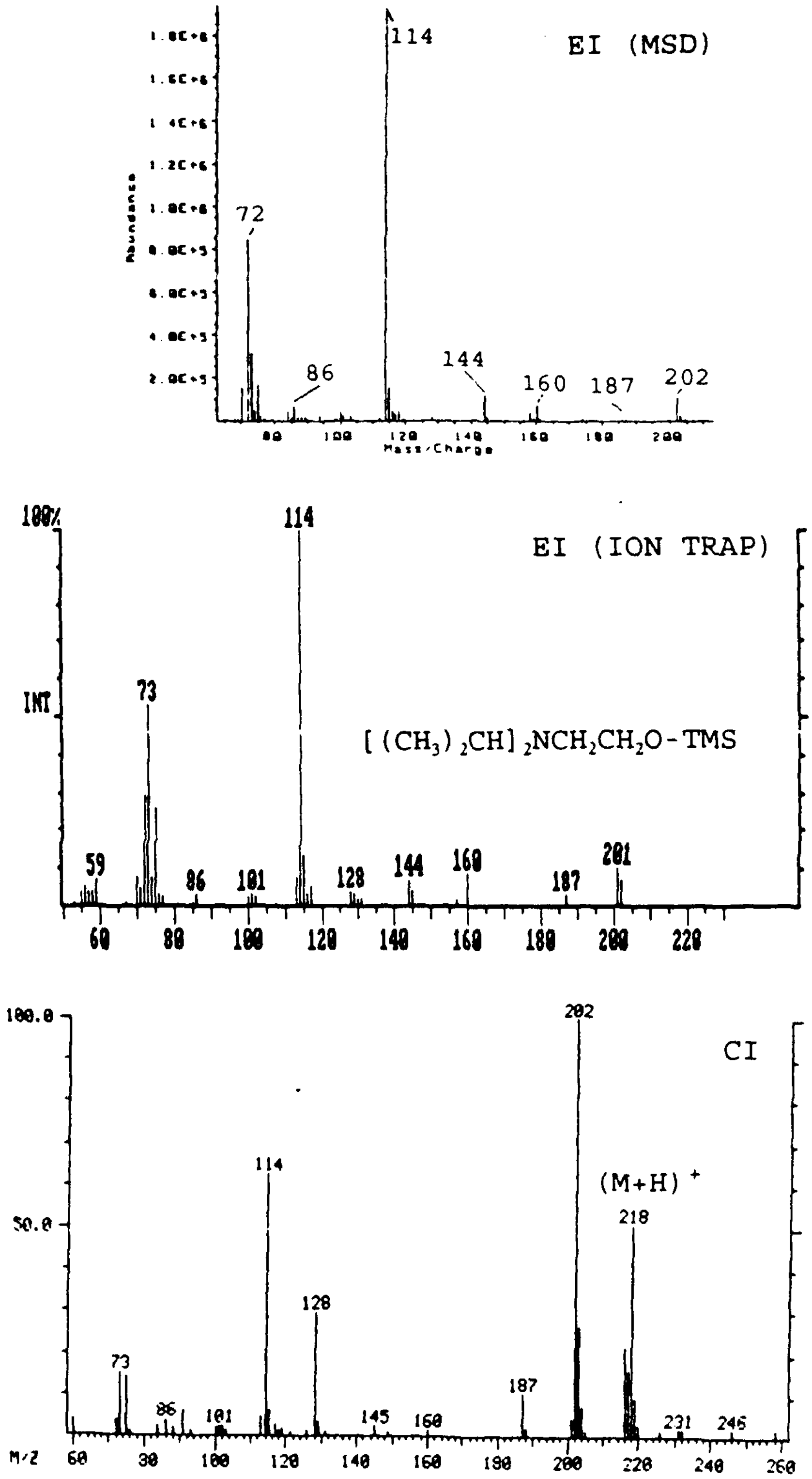


Figure E-4. EI (MSD, Ion Trap) and Methane CI Mass Spectra of 2-Diisopropylaminoethanol, TMS Derivative

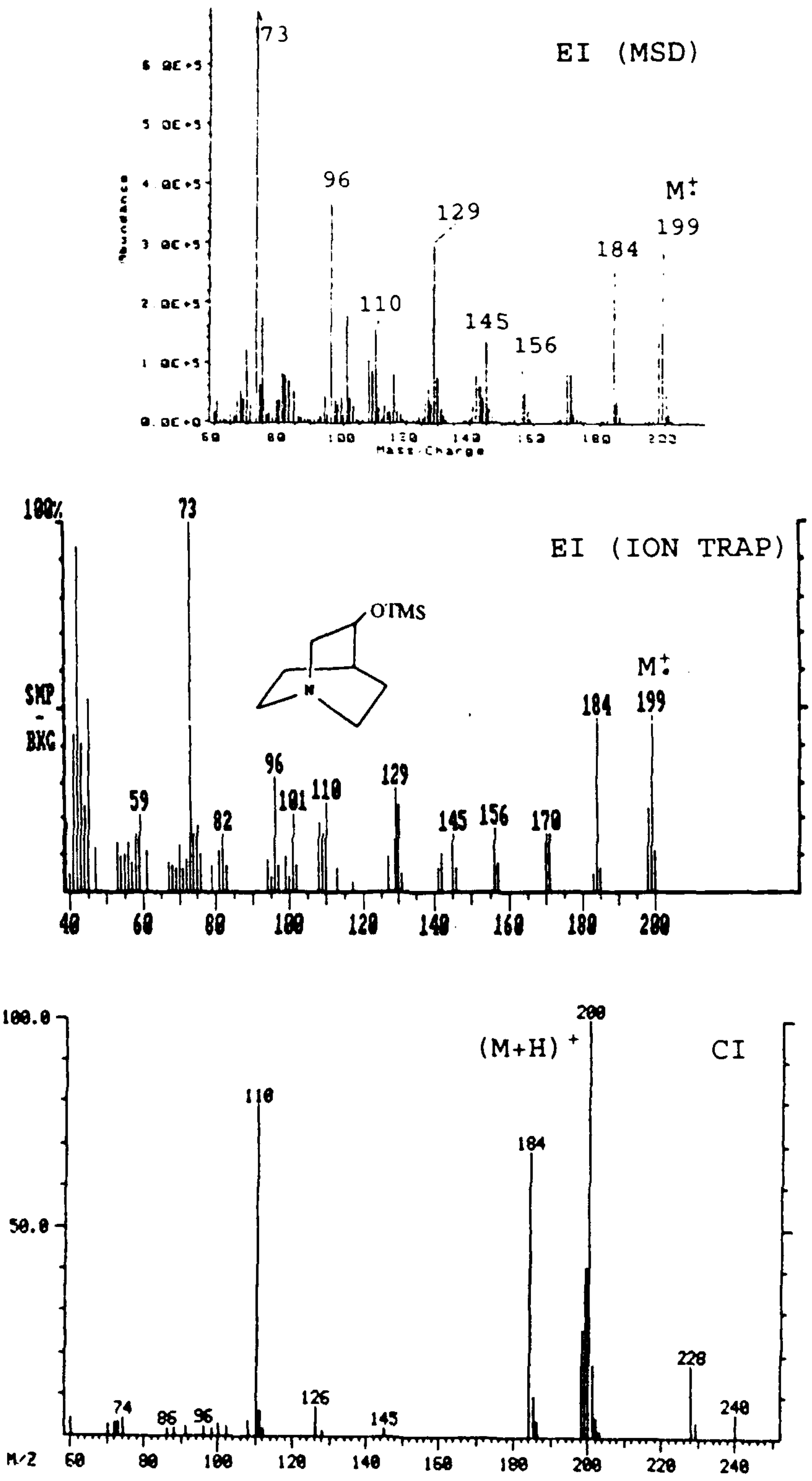


Figure E-5. EI (MSD, Ion Trap) and Methane CI Mass Spectra of 3-Quinuclidinol, TMS Derivative

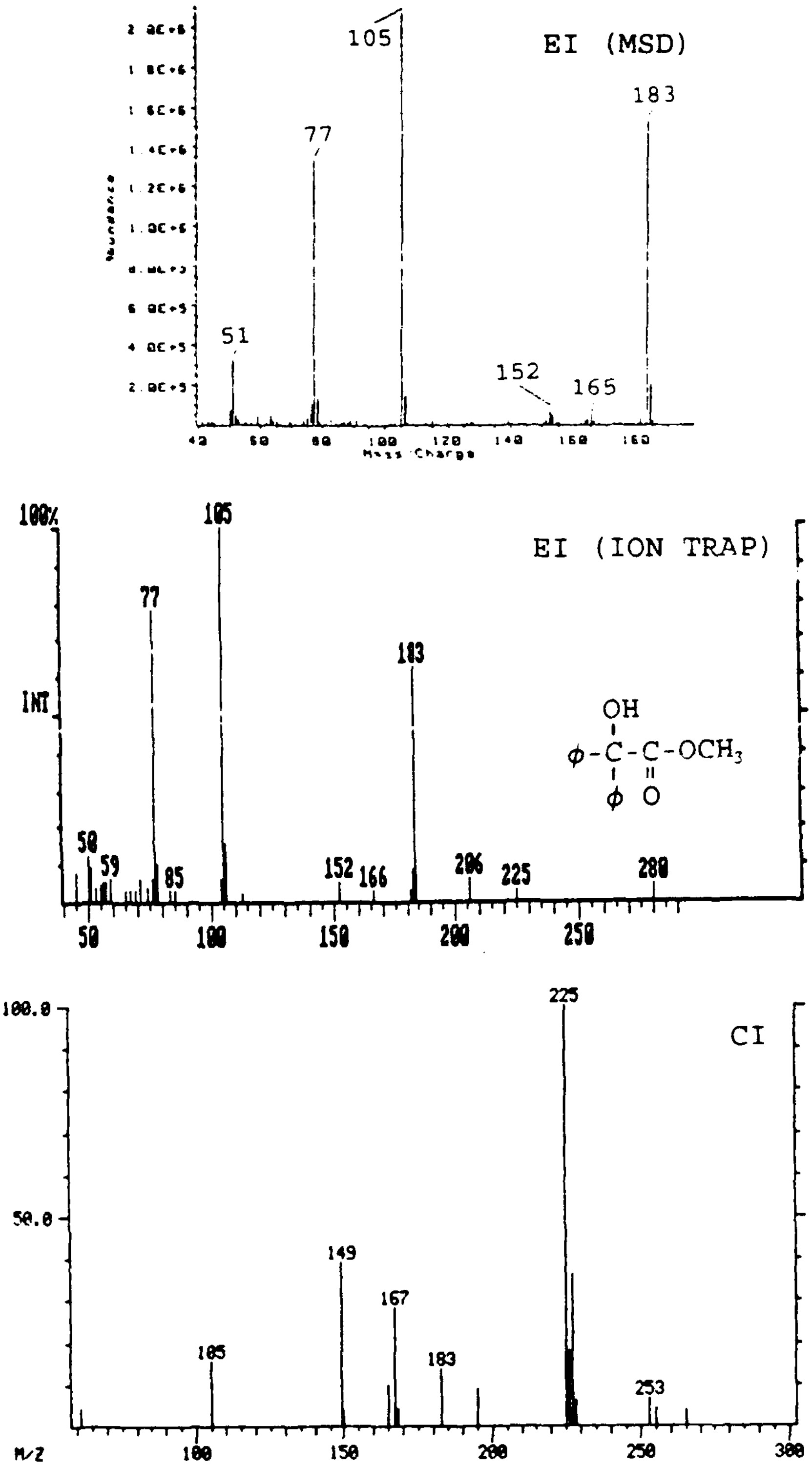


Figure E-6. EI (MSD, Ion Trap) and Methane CI Mass Spectra of Benzilic Acid, Methyl Derivative

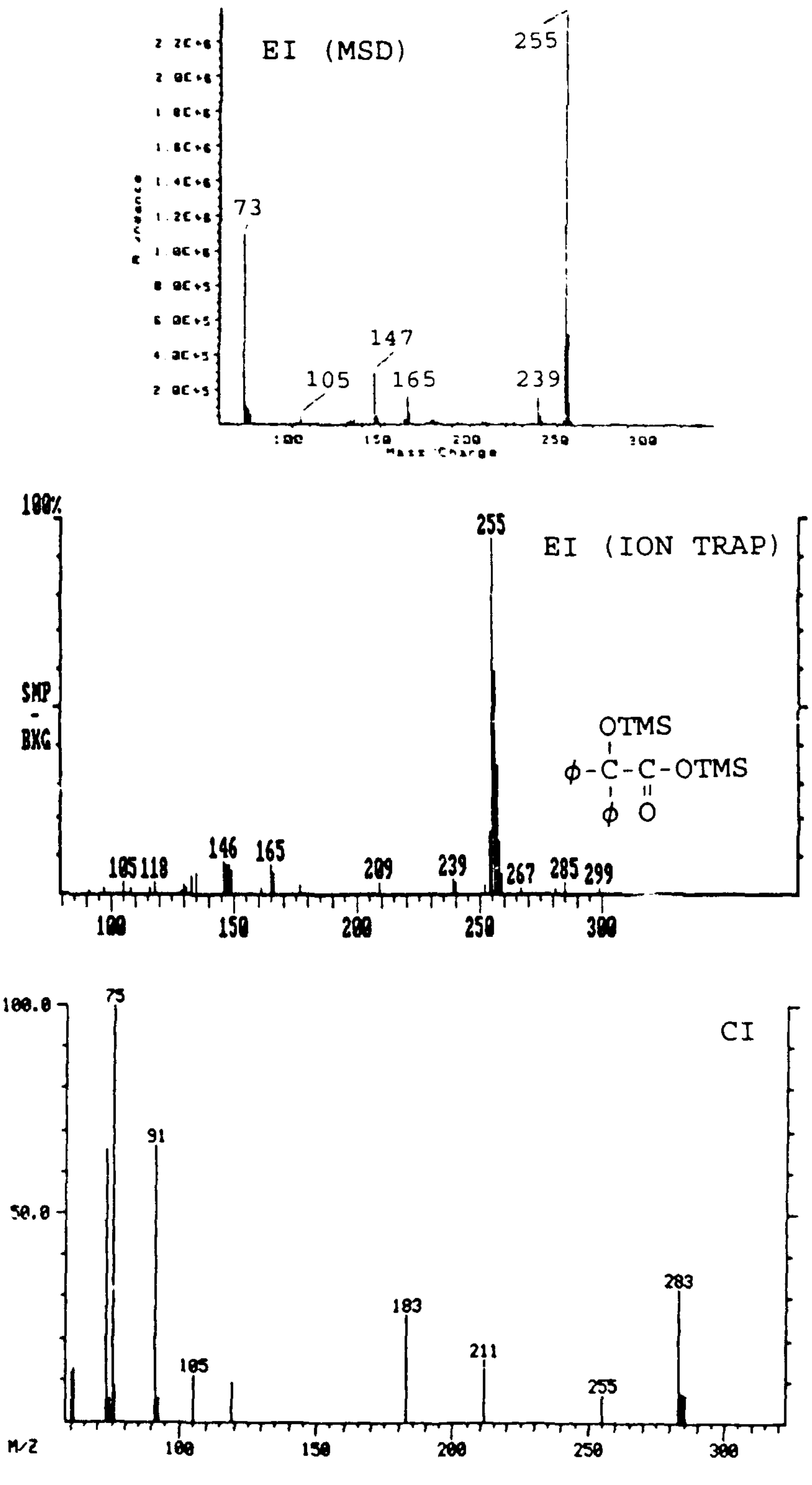


Figure E-7. EI (MSD, Ion Trap) and Methane CI Mass Spectra of Benzilic Acid, Di-TMS Derivative

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APPENDIX F

GAS CHROMATOGRAPHY METHODS AND CHROMATOGRAMS

Table F-1. GC METHOD No 1

Instrument: Hewlett-Packard 5890	COLUMN
Detector: MS (HP 5970 MSD)	Phase: DB-5
	Manufacturer: Restek
GC CONDITIONS	Length: 30 m
Carrier gas: Helium	Inner diameter: 0.25 mm
Carrier flow: 0.8 mL/min	Film thickness: 0.25 μm
Injection mode: Splitless, 60s	
Temp. program: 60 °C (3 min), 10 °C/min, 280 °C (1 min)	
Sample type analyzed: All Organic Extractions	

Table F-2. GC METHOD No 2

Instrument: Perkin-Elmer 8500	COLUMN
Detector: ITD 40 Ion Trap	Phase: RT_x-5
	Manufacturer: Restek
GC CONDITIONS	Length: 30 m
Carrier gas: Helium	Inner diameter: 0.25 mm
Carrier flow: 2 mL/min	Film thickness: 1.0 μm
Injection mode: Split/Splitless	
Temp. program: 60 °C (5 min), 10 °C/min, 250 °C (10 min)	
Sample type analyzed: All Organic Extractions	

Table F-3. GC METHOD No 3

Instrument: Finnigan 9610	COLUMN
Detector: MS (Finnigan 5100)	Phase: DB-1701
	Manufacturer: J&W Scientific
GC CONDITIONS	Length: 15 m
Carrier gas: Helium	Inner diameter: 0.25 mm
Carrier flow: 1 mL/min	Film thickness: 0.25 μm
Injection mode: Split/Splitless	
Temp. program: 60 °C (1 min), 10 °C/min, 280 °C (10 min)	
Sample type analyzed: All Organic Extractions	

Table F-4. GC METHOD No 4

Instrument: Hewlett-Packard 5880	COLUMN
Detector: Flame Ionization	Phase: RT_x-5
	Manufacturer: Restek
GC CONDITIONS	Length: 30 m
Carrier gas: Helium	Inner diameter: 0.53 mm
Carrier flow: 10 mL/min	Film thickness: 1.0 μm
Injection mode: Vaporization Liner	
Temp. program: 60 °C (3 min), 10 °C/min, 250 °C (5 min)	
Sample type analyzed: All Organic Extractions	

Table F-5. GC METHOD No 5

Instrument: Varian 6000	COLUMN
Detector: FPD (P-Mode)	Phase: RT_x-35
	Manufacturer: Restek
GC CONDITIONS	Length: 30 m
Carrier gas: Helium	Inner diameter: 0.53 mm
Carrier flow: 10 mL/min	Film thickness: 1.0 μm
Injection mode: On Column	
Temp. program: 60 °C (3 min), 10 °C/min, 250 °C (5 min)	
Sample type analyzed: All Organic Extractions	

Table F-6. GC METHOD No 6

Instrument: Perkin-Elmer 8500	COLUMN
Detector: FPD (S-Mode)	Phase: RT_x-5
	Manufacturer: Restek
GC CONDITIONS	Length: 30 m
Carrier gas: Helium	Inner diameter: 0.53 m
Carrier flow: 10 mL/min	Film thickness: 1.0 μm
Injection mode: On Column	
Temp. program: 60 °C (3 min), 10 °C/min, 250 °C (5 min)	
Sample type analyzed: All Organic Extractions	

Table F-7. GC METHOD No 7

Instrument: Finnigan 9610	COLUMN
Detector: MS (Finnigan 5100)	Phase: AT-5
	Manufacturer: Alltech
GC CONDITIONS	Length: 25 m
Carrier gas: Helium	Inner diameter: 0.25 mm
Carrier flow: 1 mL/min	Film thickness: 0.20 μm
Injection mode: Splitless, 15s	
Temp. program: 40 °C (1 min), 10 °C/min, 280 °C (10 min)	
Sample type analyzed: All Organic Extractions	

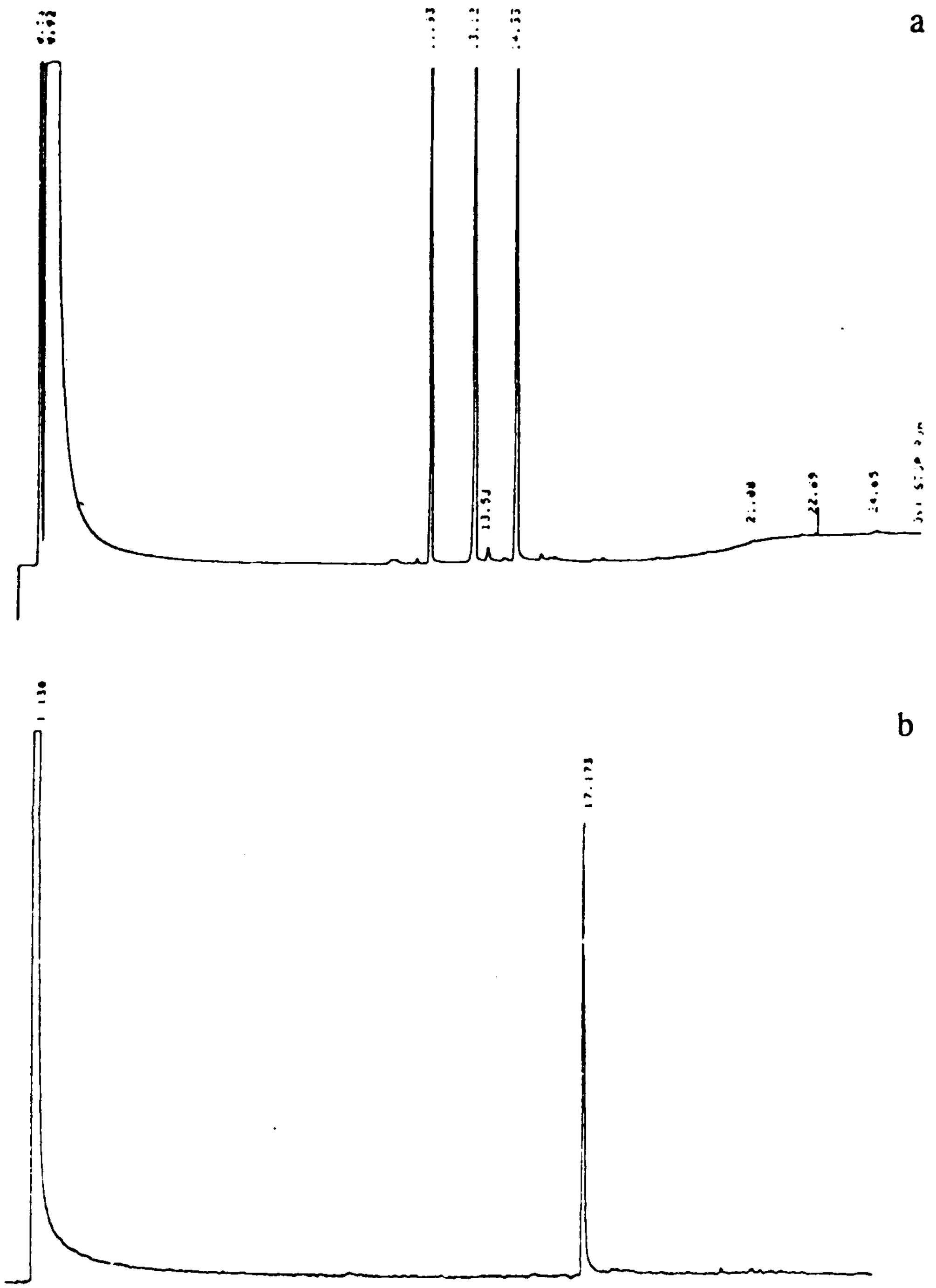


Figure F-1. GC Chromatograms of a) FID Test Mix, solution of 0.033% (W/W) Each of n-Tetradecane, n-Pentadecane, n-Hexadecane in Hexane (Method GC4) and b) FPD Test Mix), 1 PPM Tributyl Phosphate (Method GC5)

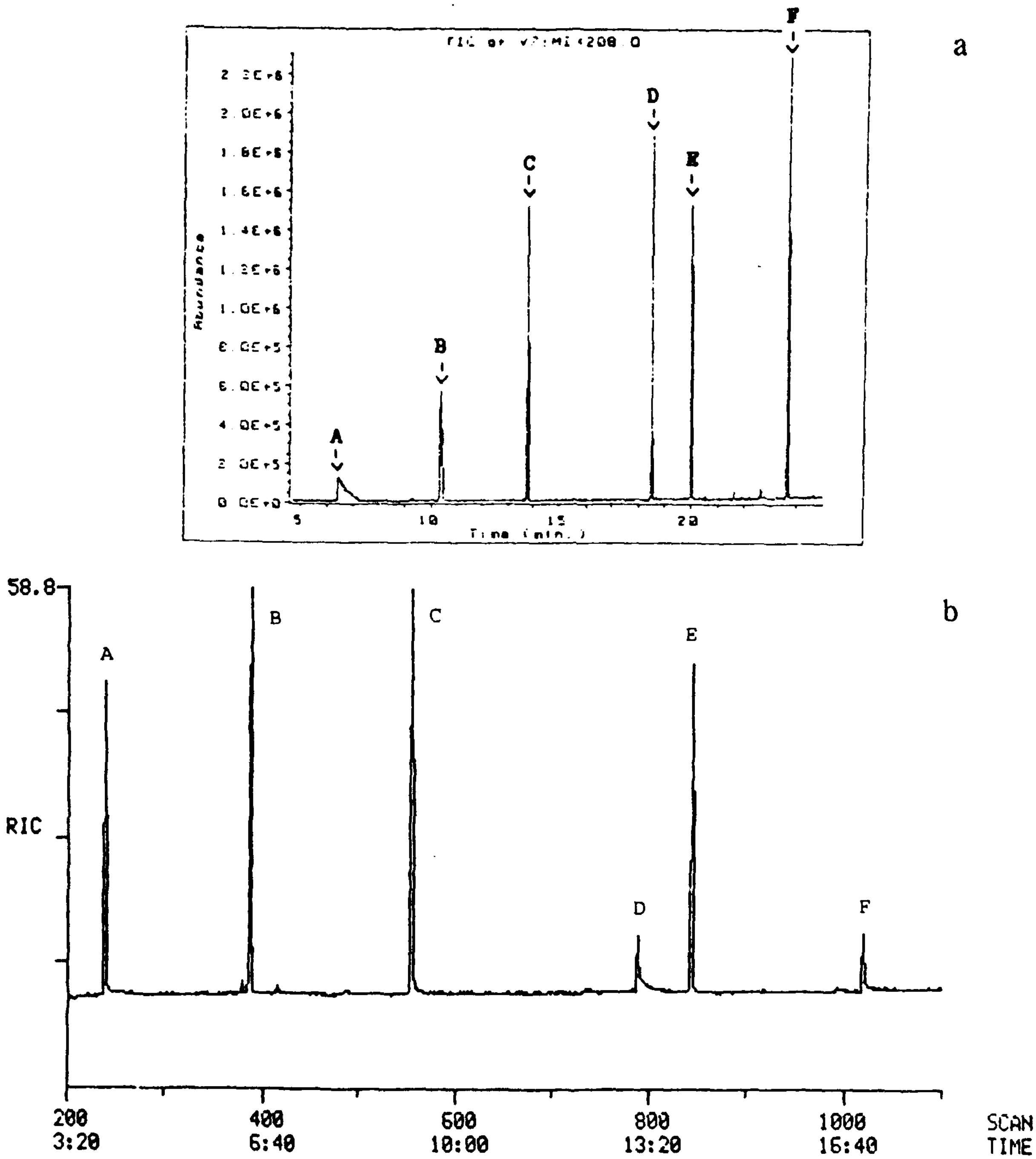
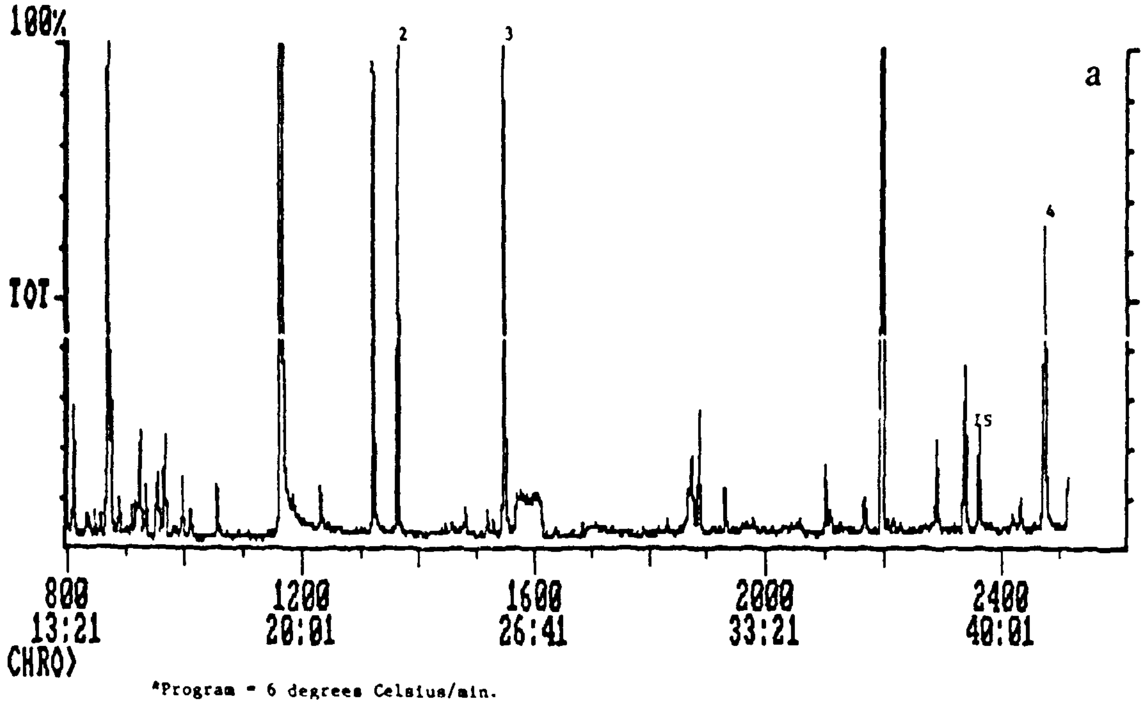


Figure F-2. a) GC/MSD/EI (Method GC1/MS1) and b) GC/MS/CI (Method GC3/MS3) Chromatograms of Test Mix Containing 20 PPM Each of Trimethyl Phosphate (A), 2,6-Dimethylphenol (B), 5-Chloro-2-methylaniline (C), Tributyl Phosphate (D), Dibenzothiophene (E) and Methyl Stearate (F).



*Program = 6 degrees Celsius/min.

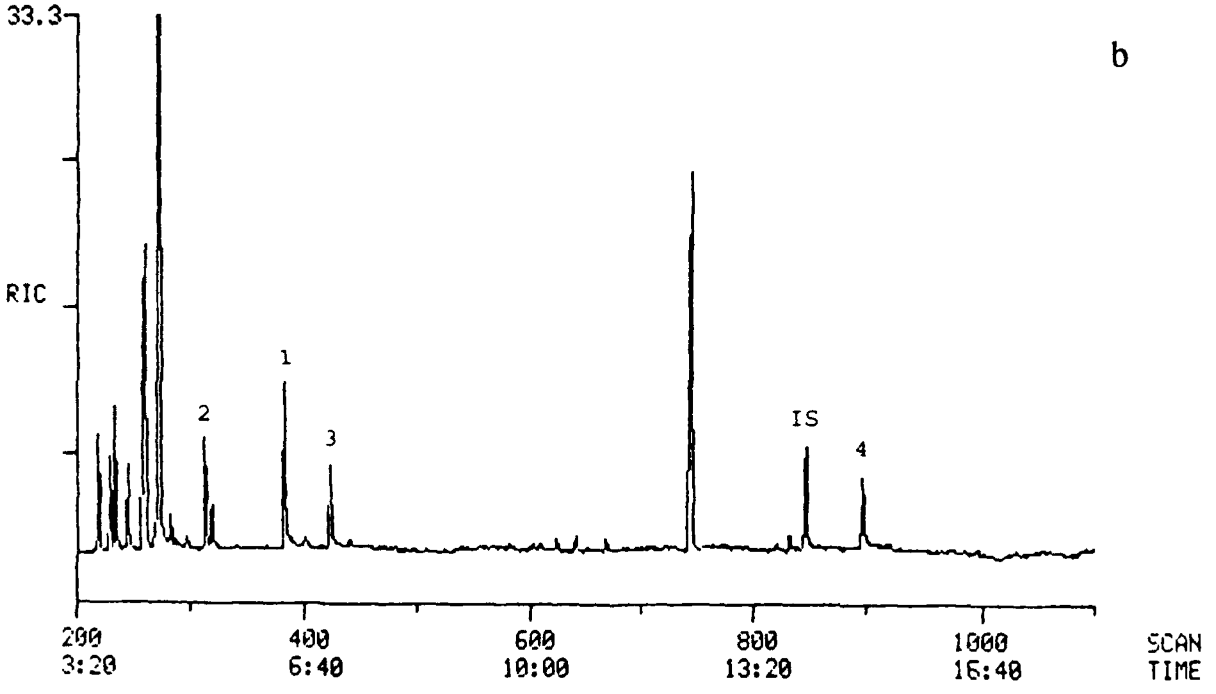


Figure F-3. a) GC/ITD/EI (Method GC2/MS2) and b) GC/MS/CI (Method GC3/MS3) Chromatograms of TMS Derivative of Authentic Standard Containing 50 PPM Each of MPA (1), DIAE (2), 3-Q (3), BA (4) and Internal Standard Dibenzothiophene (IS) in Methanol.

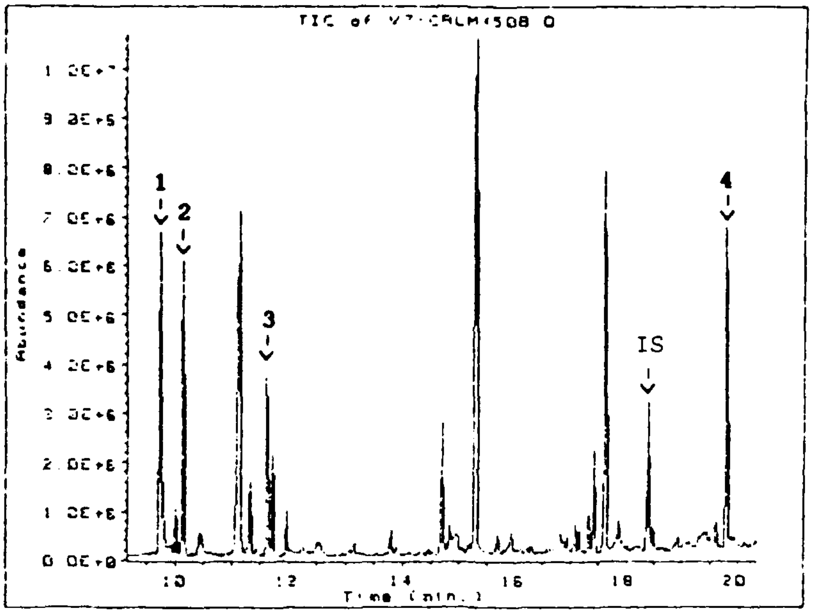


Figure F-4. GC/MSD/EI (Method GC1/MS1) Chromatogram of TMS Derivative of Authentic Standard Containing 50 PPM Each of MPA (1), DIAE (2), 3Q (3), BA (4) and DBT (IS).

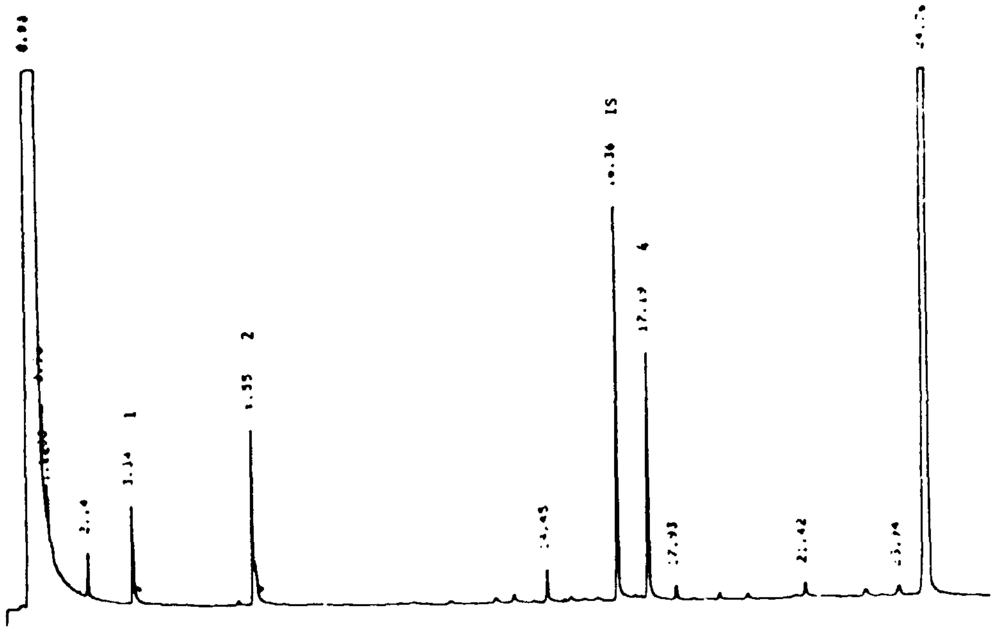
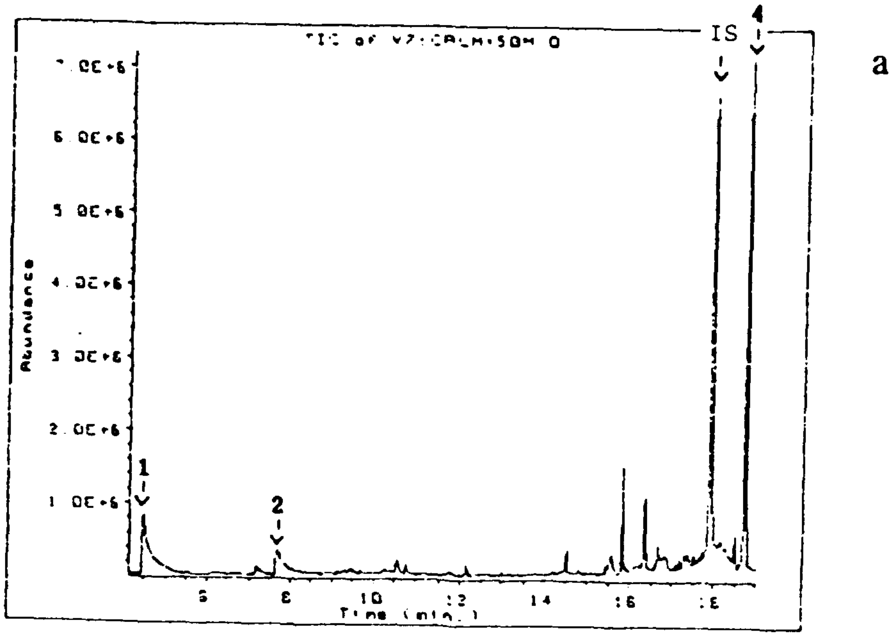


Figure F-5. GC/FID (Method GC4) Chromatogram of Methyl Derivative of Authentic Standard Containing 50 PPM Each of MPA (1), DIAE (2), 3Q (3), BA (4) and DBT (IS).



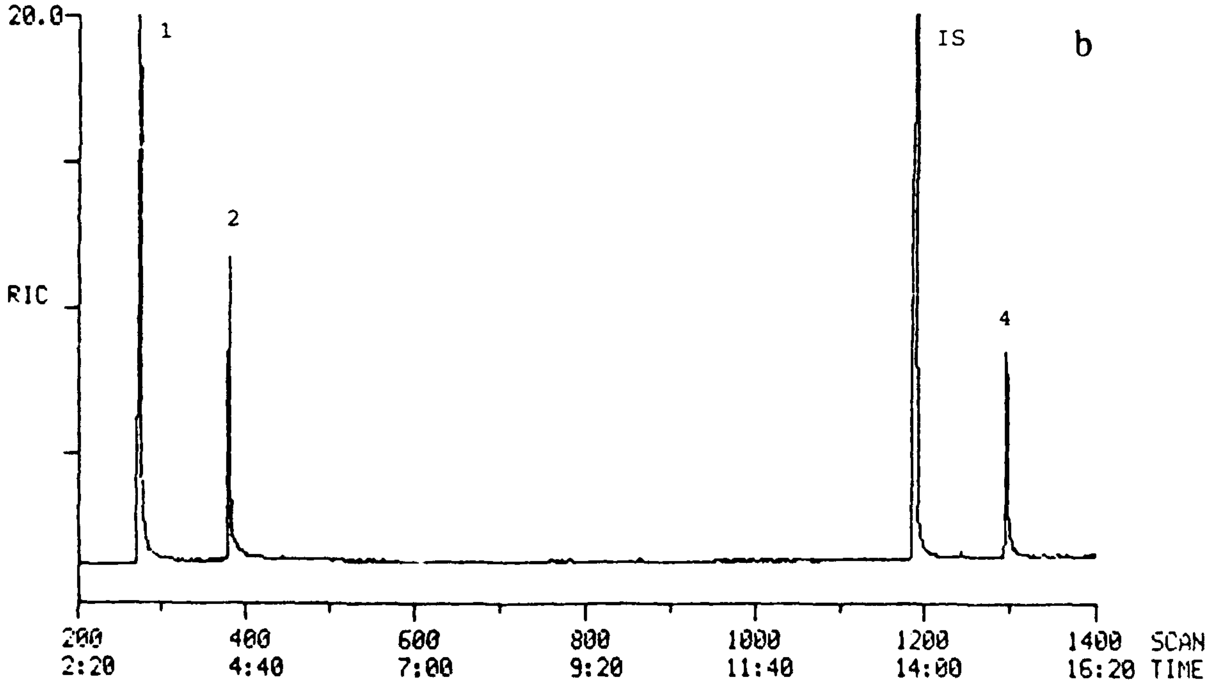


Figure F-6. a) GC/MSD/EI (Method GC1/MS1) and b) GC/MS/CI/SIM (Method GC3/MS3) Chromatograms of Methyl Derivative of Authentic Standard Containing 50 PPM Each of MPA (1), DIAE (2), 3-Q (3), BA (4) and DBT (IS).

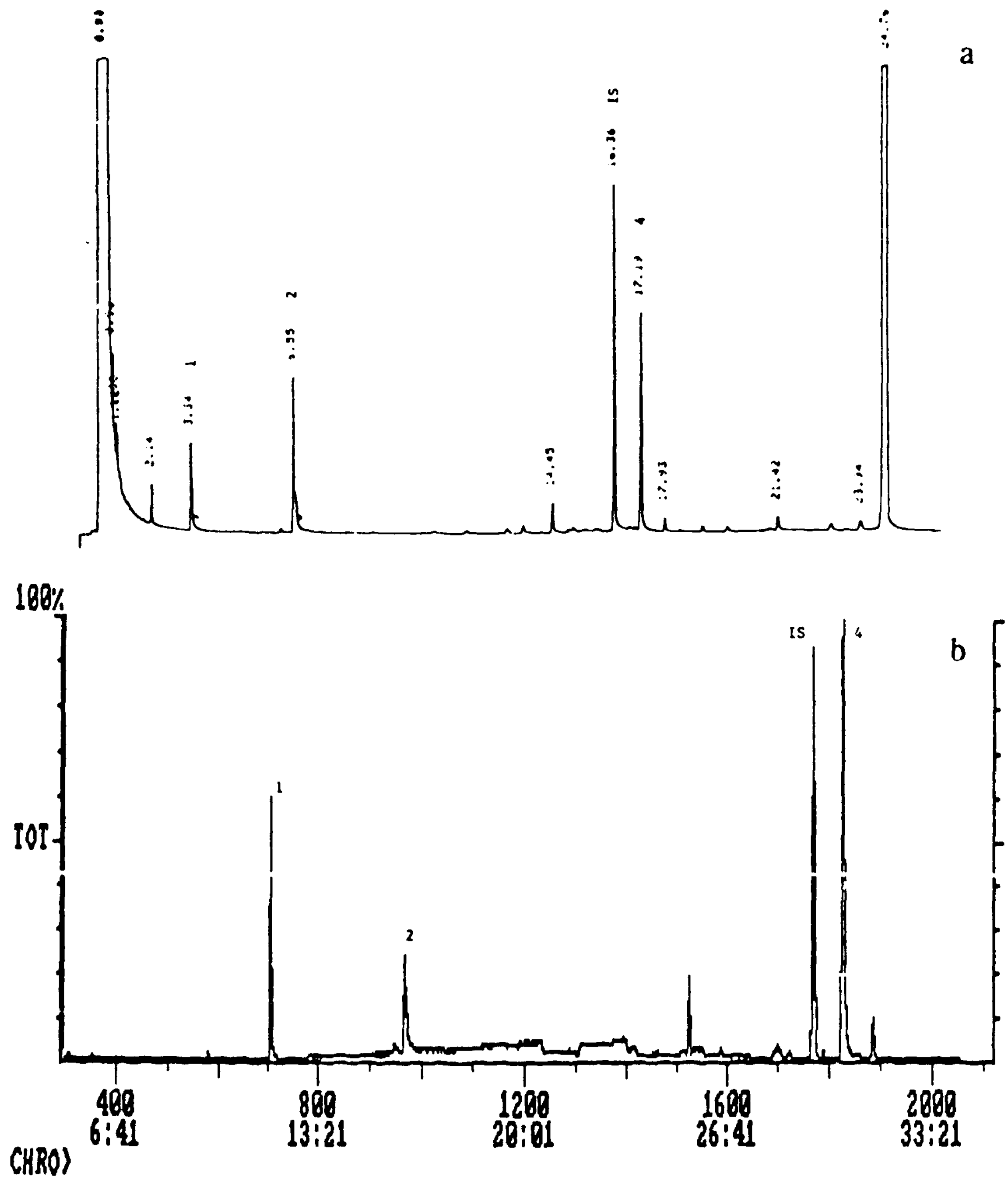


Figure F-7. a) GC/FID (Method GC4) and b) GC/ITD/EI (Method GC2/MS2) Chromatograms of Methyl Derivative of Authentic Standard Containing 50 PPM Each of MPA (1), DIAE (2), 3-Q (3), BA (4) and DBT (IS).

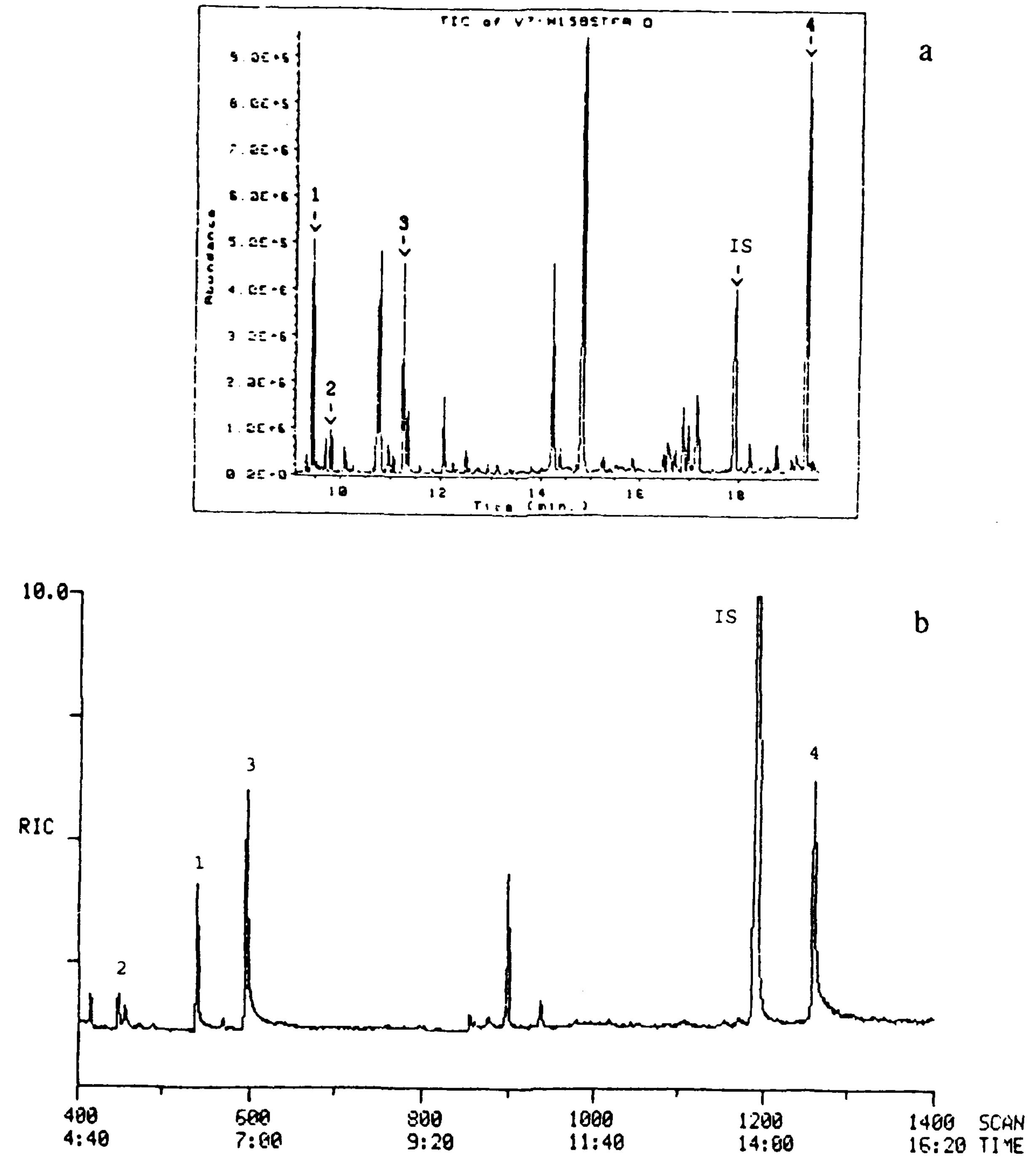


Figure F-8. a) GC/MS/EI (Method GC1/MS1) and b) GC/MS/CI/SIM (Method GC3/MS3) Chromatograms of Water Sample W-15 (1 mL, Acidified, TMS Derivative). Peak Numbers are Identified in Table 2.

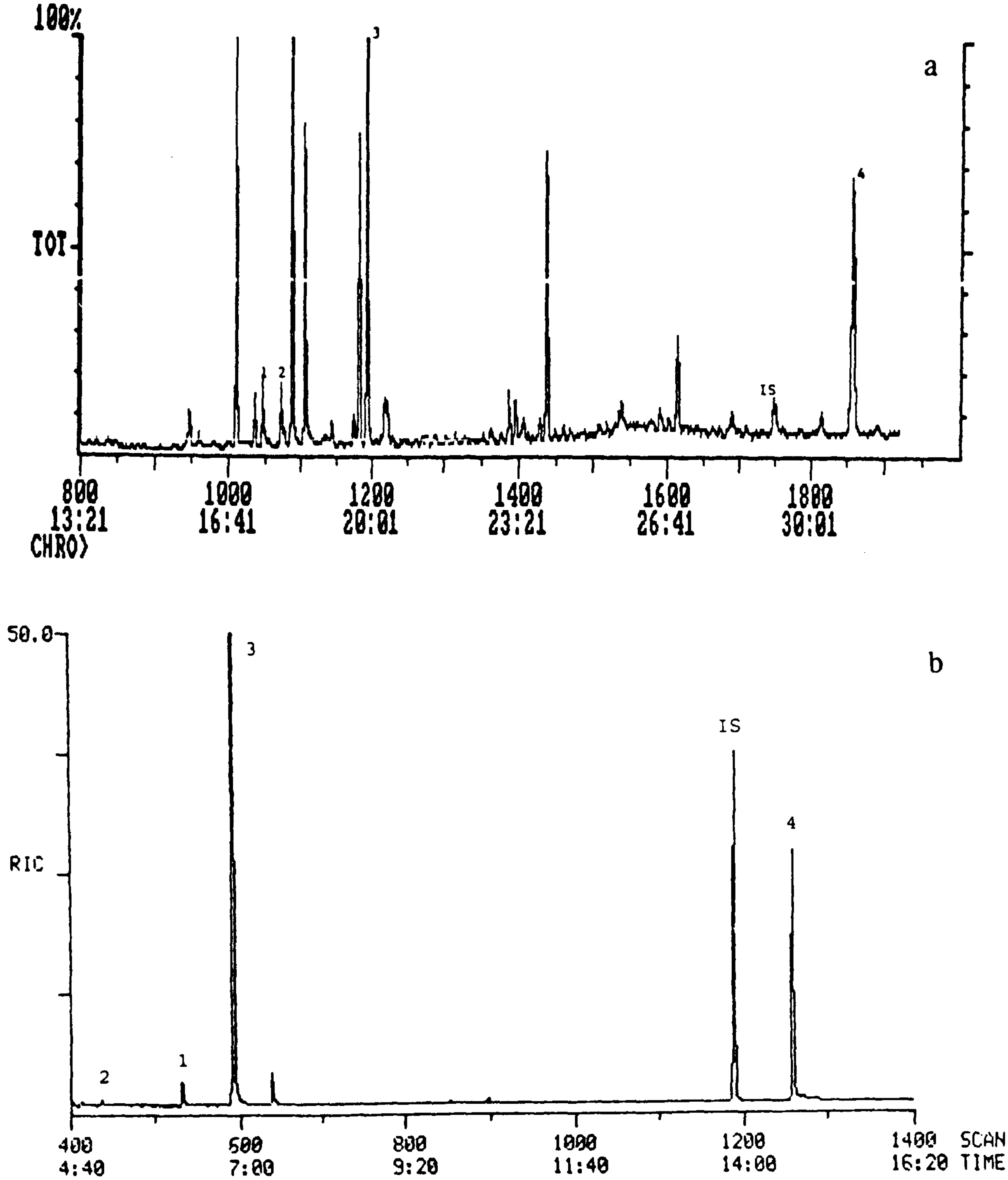


Figure F-9. a) GC/ITD/EI (Method GC2/MS2) and b) GC/MS/CI/SIM (Method GC3/MS3) Chromatograms of NMR Water Sample W-15C (TMS Derivative). Peak Numbers are Identified in Table 2.

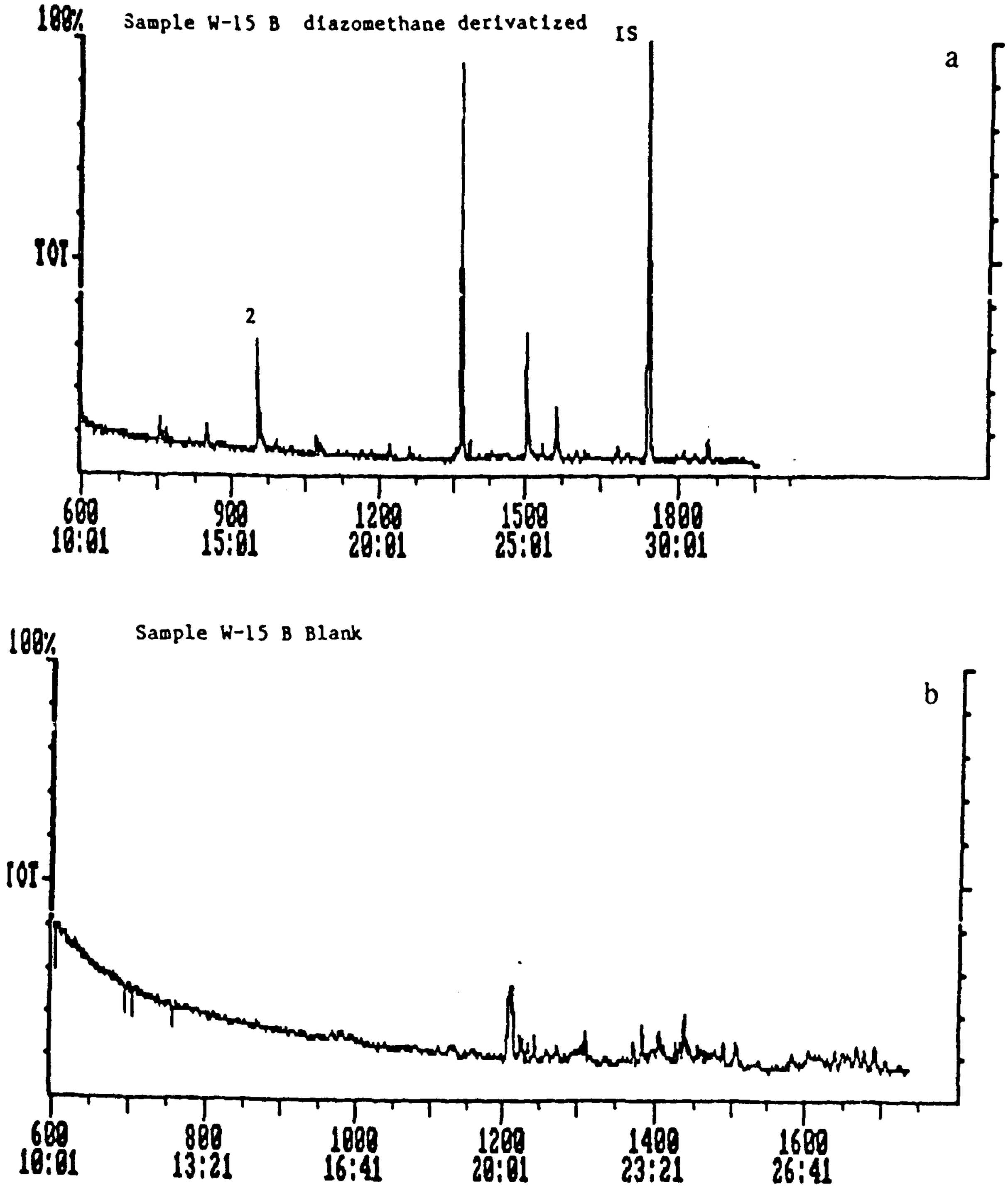


Figure F-10. GC/ITD/EI (Method GC2/MS2) Chromatograms of Water Sample W-15 Neutral Extract (W-15B*) and b) Water Blank WB-15 Neutral Extract (WB-15B*), Methyl Derivative. Peak Numbers are Identified in Table 2.

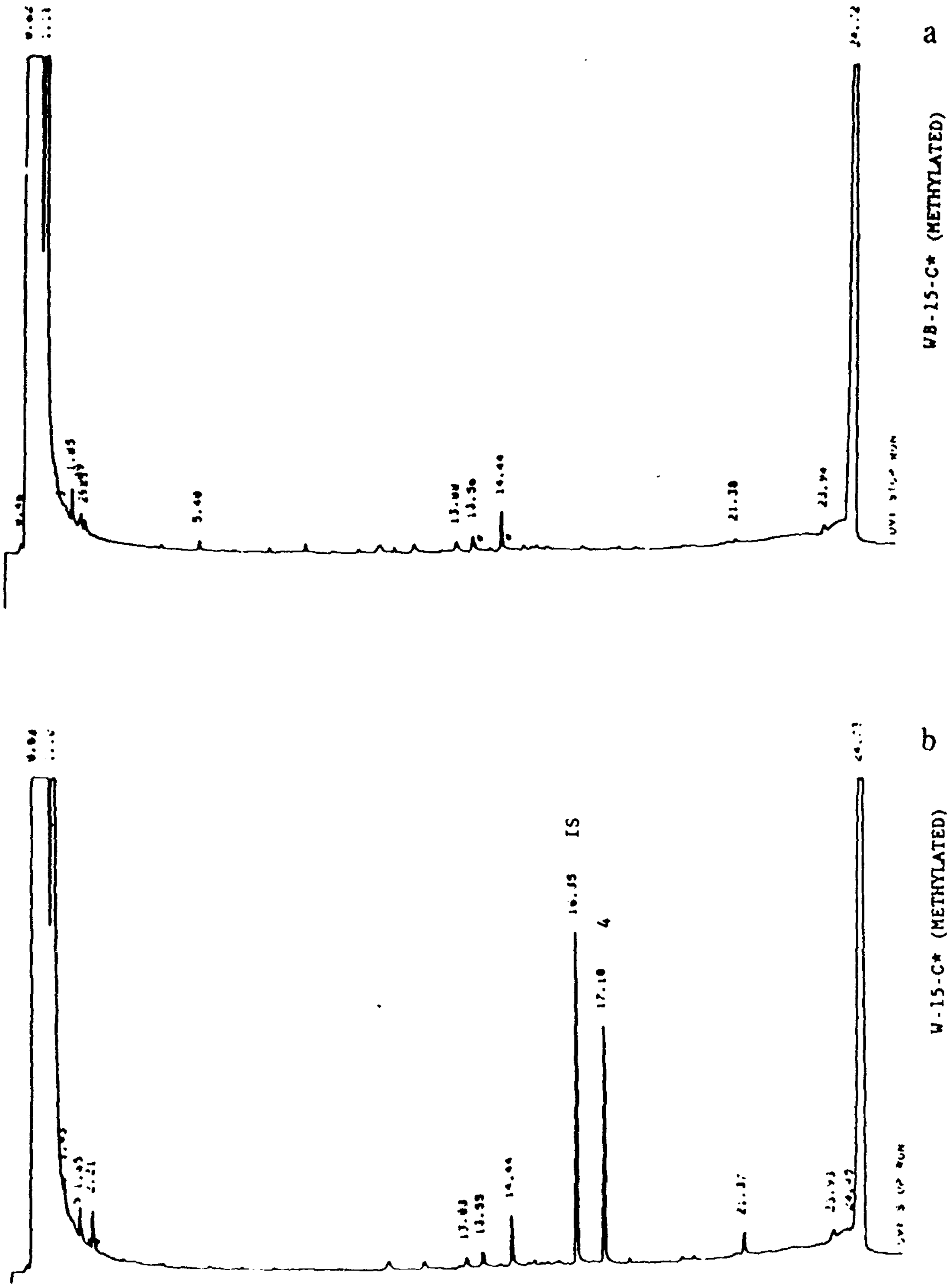


Figure F-11. GC/FID (Method GC4) Chromatograms of Water Sample W-15 Acidic Extract (W-15C*) and b) Water Blank WB-15 Acidic Extract (WB-15C*), Methyl Derivative. Peak Numbers are Identified in Table 2.

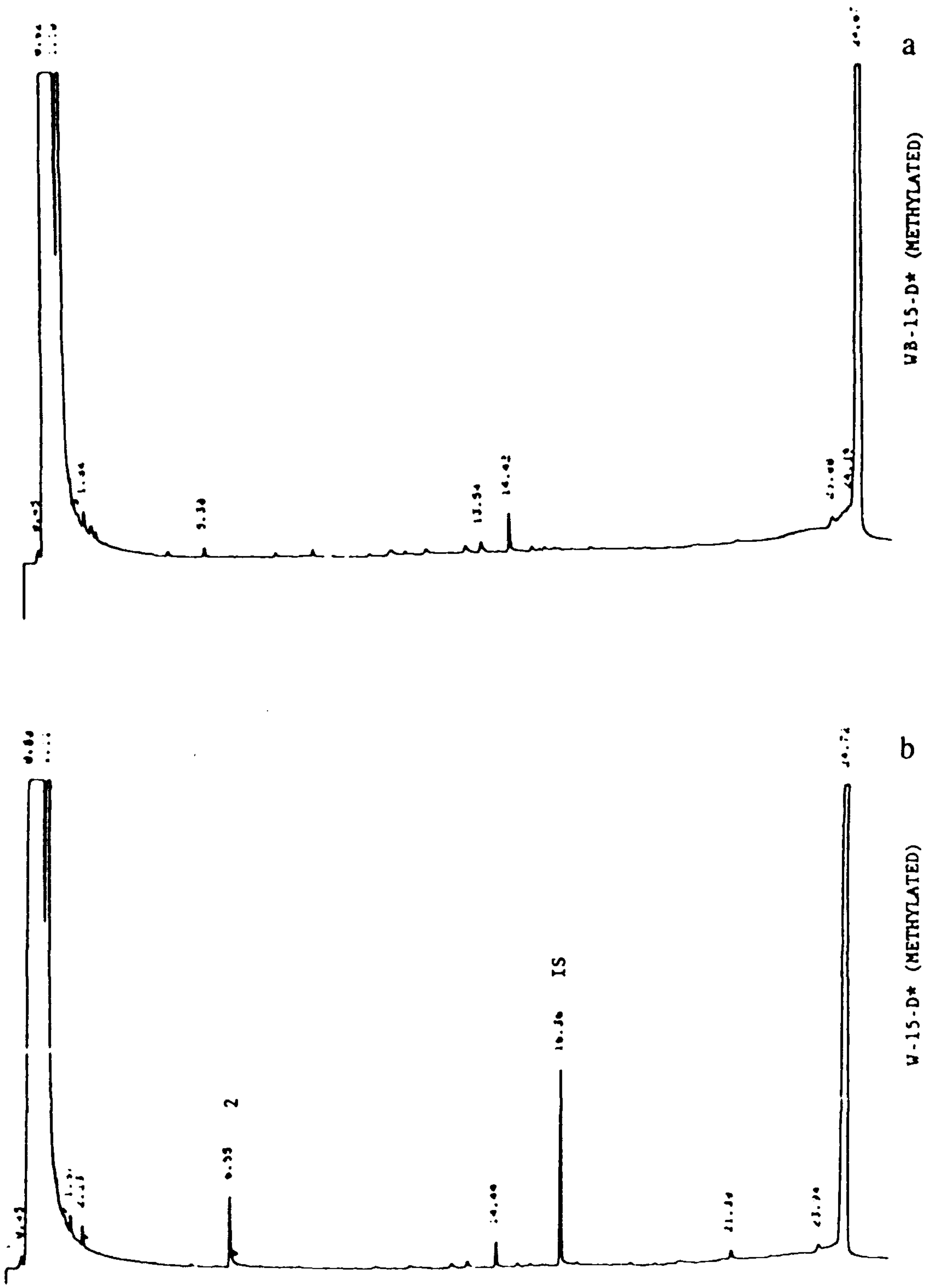


Figure F-12. GC/FID (Method GC4) Chromatograms of Water Sample W-15 Basic Extract (W-15D*) and b) Water Blank WB-15 Basic Extract (WB-15D*), Methyl Derivative. Peak Numbers are Identified in Table 2.

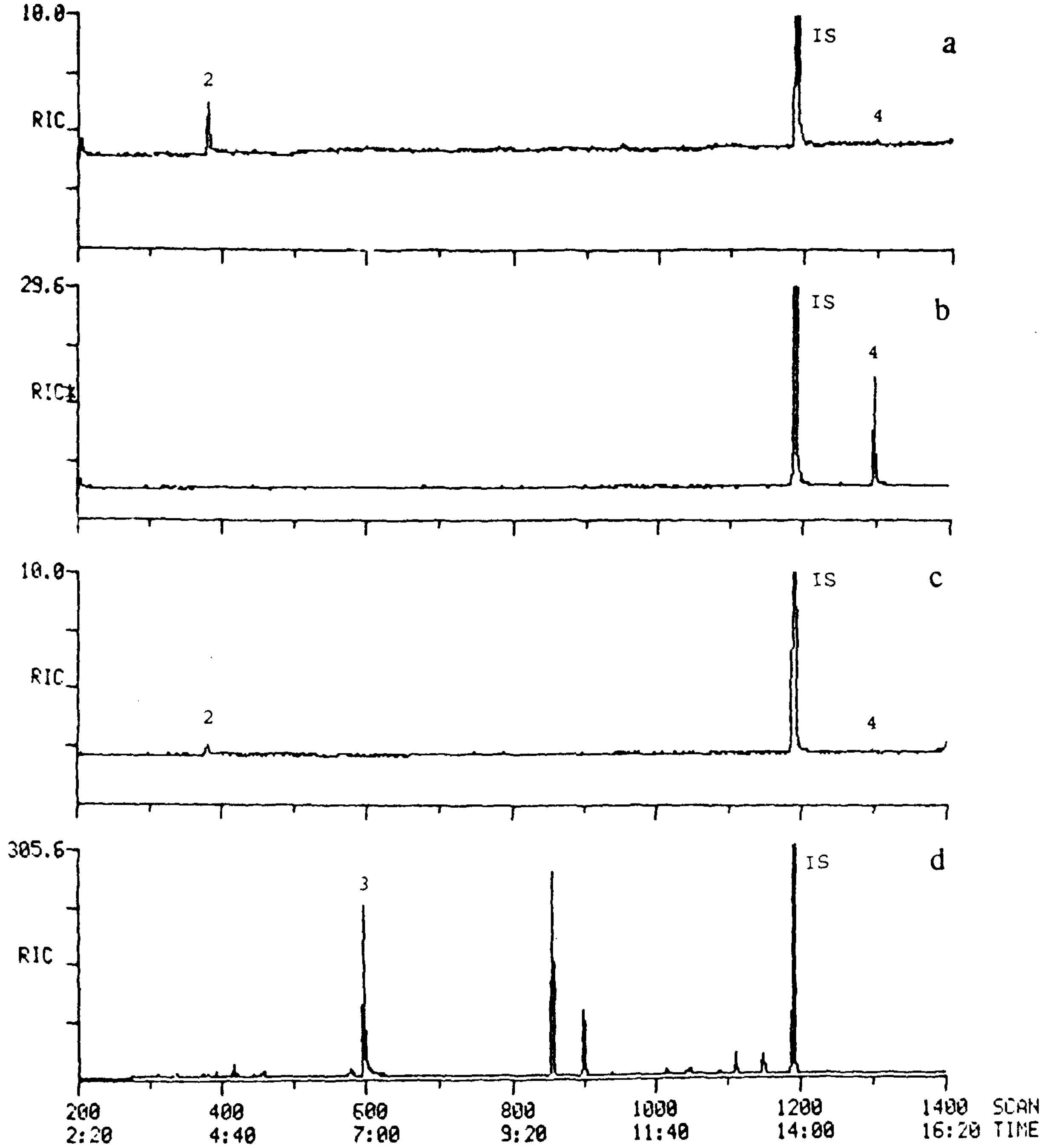


Figure F-13. GC/MS/CI/SIM (Method GC3/MS3) Chromatograms of Water Sample W-15 a) Neutral Extract B* (Methylated), b) Acidic Extract C* (Methylated), c) Basic Extract C* (Methylated) and d) Residue E* (TMS Derivative). Peak Numbers are Identified in Table 2.

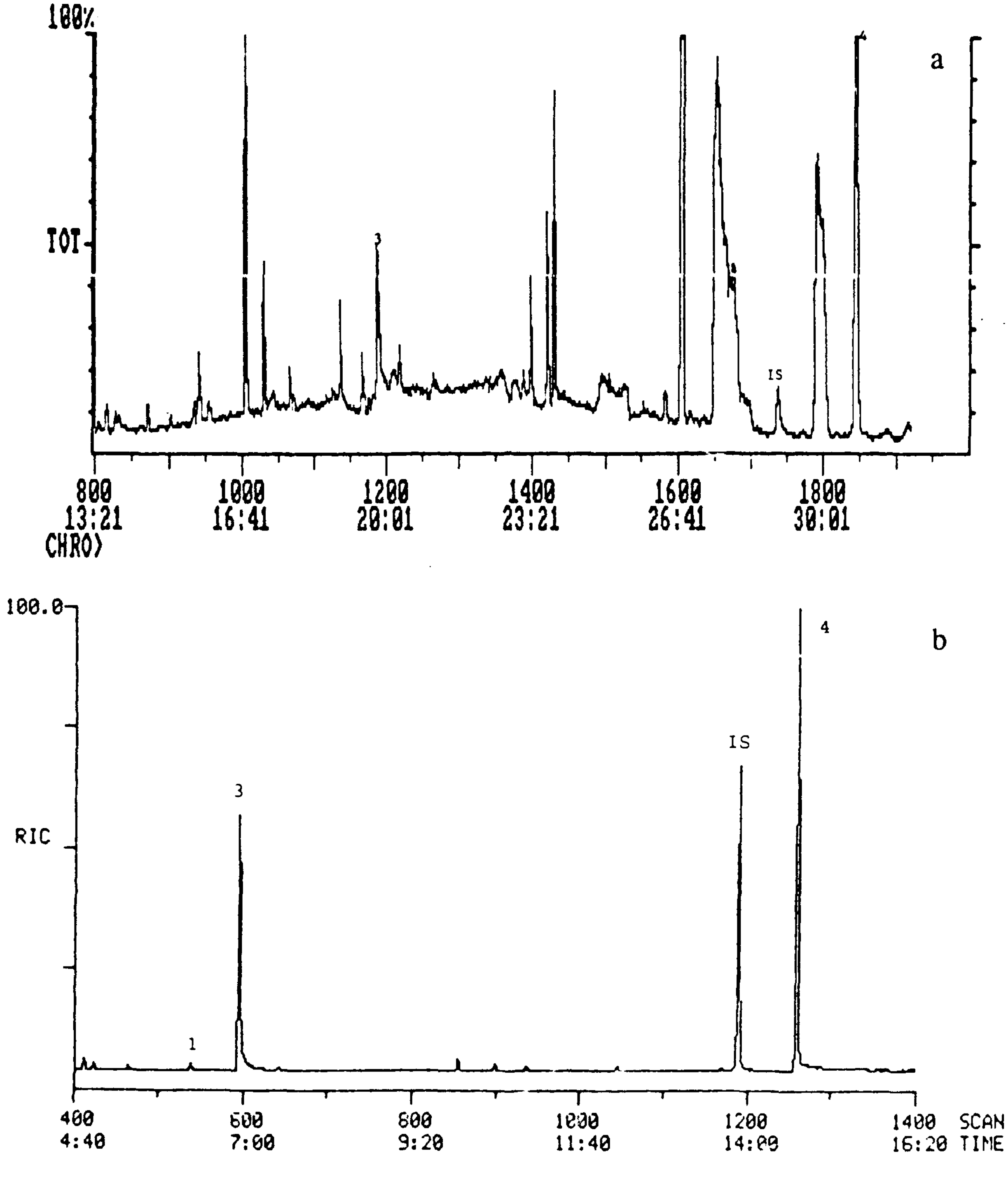


Figure F-14. a) GC/ITD/EI (Method GC2/MS2) and b) GC/MS/CI/SIM (Method GC3/MS3) Chromatograms of NMR Water Sample KEW-14C, TMS Derivative. Peak Numbers are Identified in Table 2.

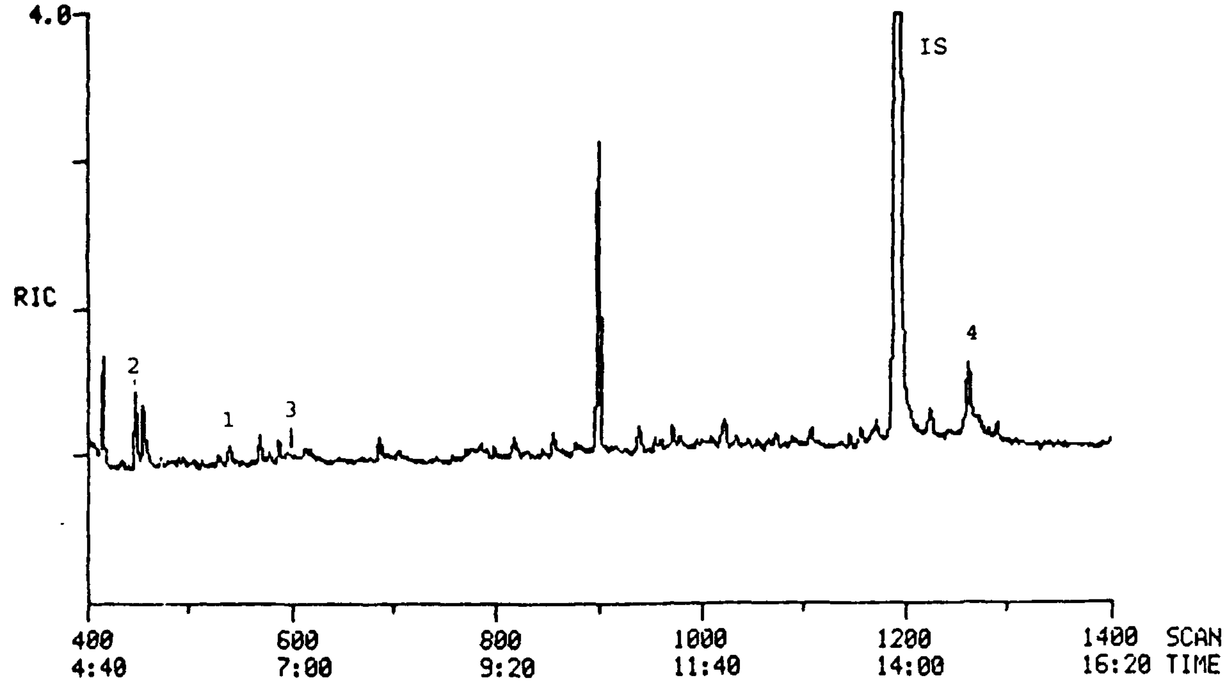


Figure F-15. GC/MS/CI/SIM (Method GC3/MS3) Chromatogram of Water/ Methanol Extract of Soil Sample SA-15 (1.5 mL), TMS Derivative. Peak Numbers are Identified in Table 2.

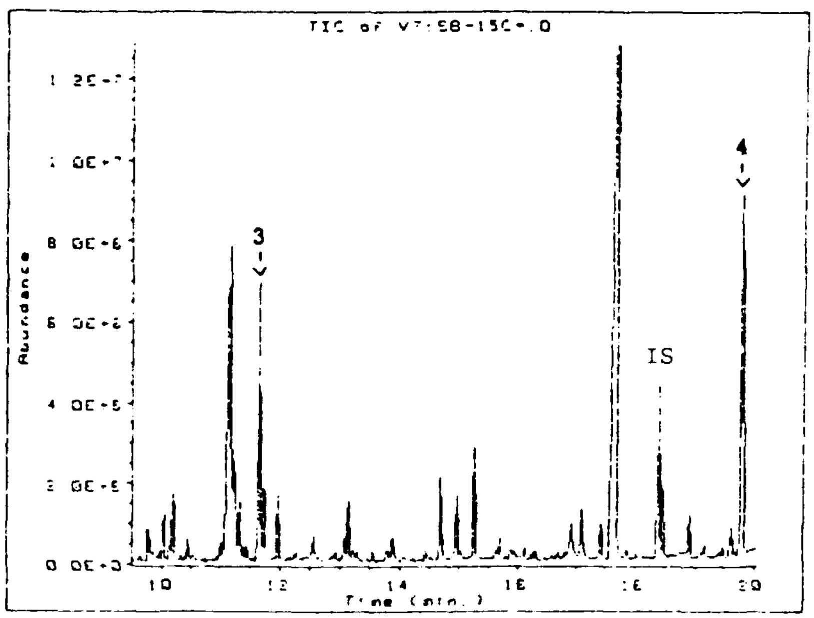


Figure F-16. GC/MSD/EI (Method GC1/MS1) Chromatogram of Water Extract SB-15C* of Soil Sample SB-15, TMS Derivative. Peak Numbers are Identified in Table 2.

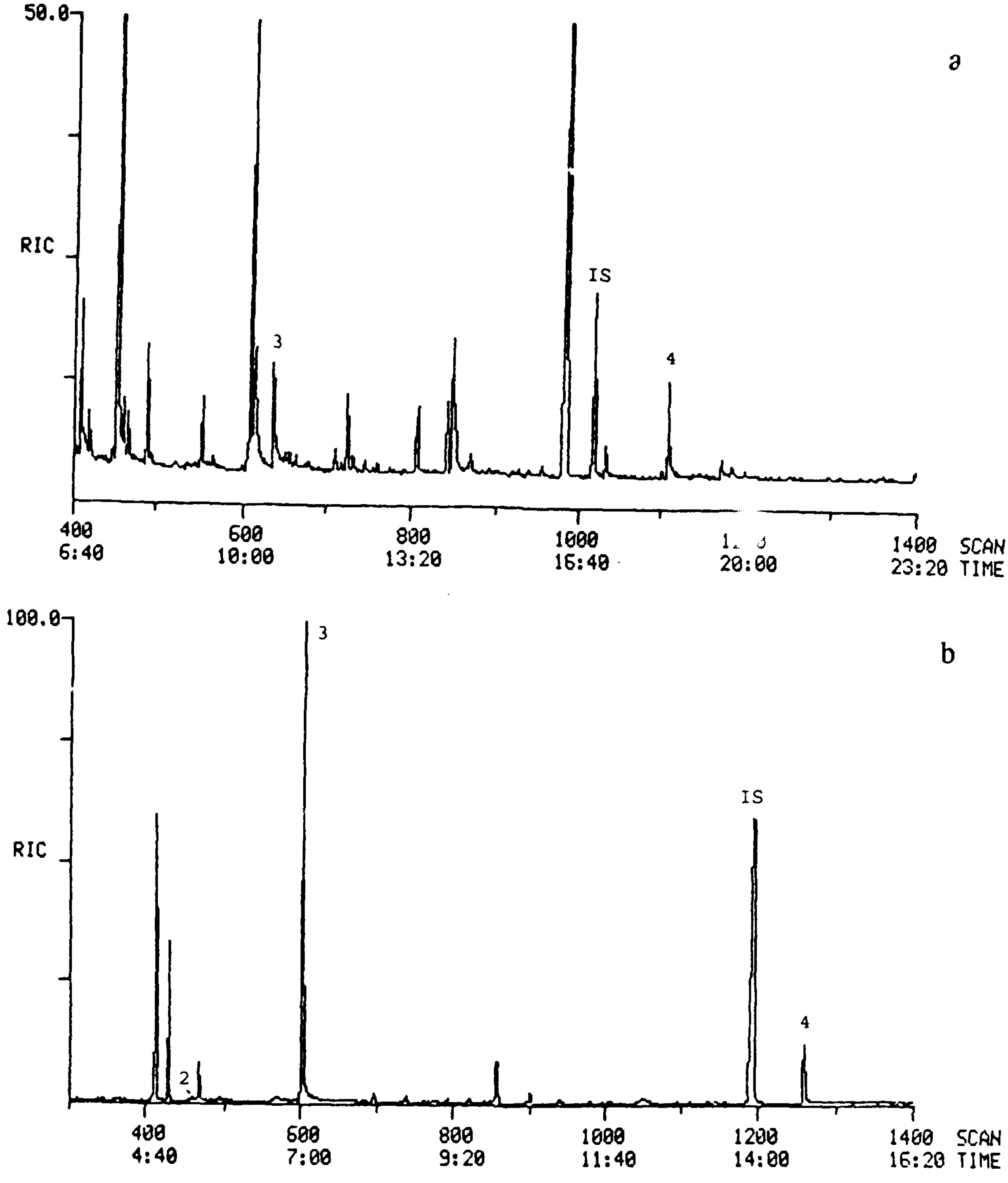


Figure F-17. GC/MS/CI a) Full Scan (Method GC7/MS3) and b) SIM (Method GC3/MS3) Chromatograms of Water Extract SB-15C* of Soil Sample SB-15, TMS Derivative. Peak Numbers are Identified in Table 2.

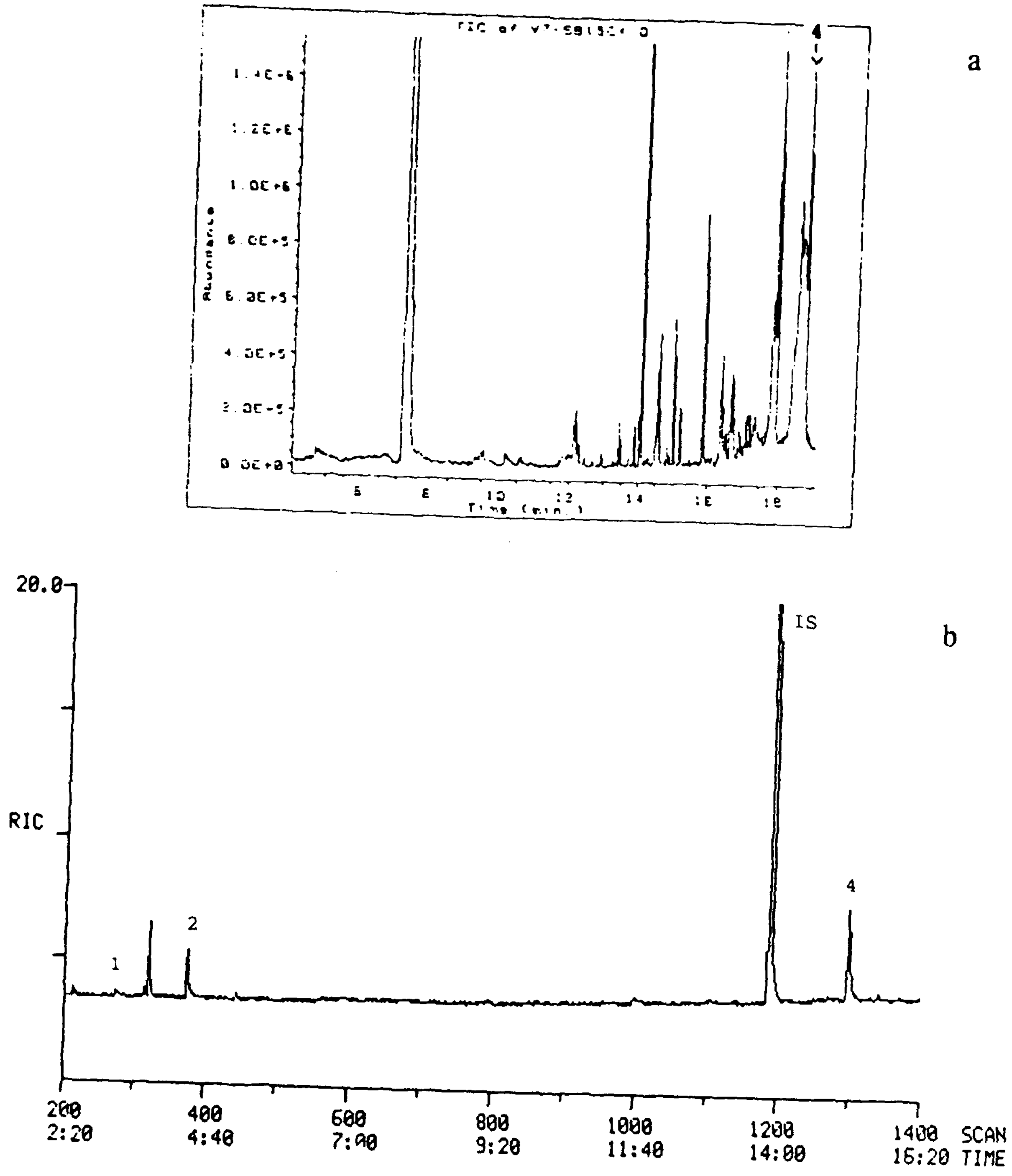


Figure F-18. GC/MSD/EI (Method GC1/MS1) and b) GC/MS/CI/SIM (Method GC3/MS3) Chromatograms of Water Extract SB-15C* of Soil Sample SB-15, Methyl Derivative. Peak Numbers are Identified in Table 2.

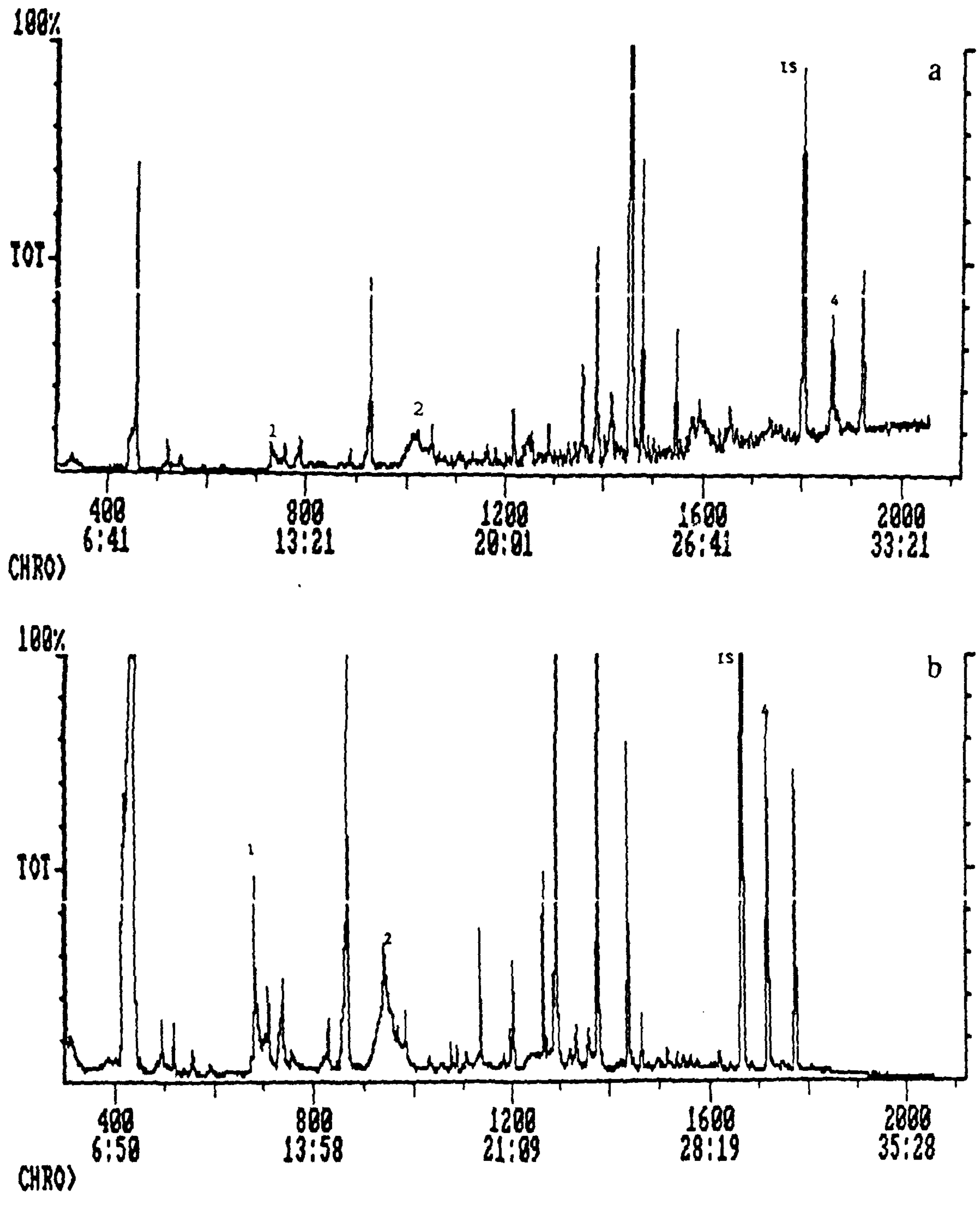


Figure F-19. GC/ITD a) EI (Method GC2/MS2) and b) CI (Method GC2/MS4) Chromatograms of Water Extract SN-15C* of Soil Sample SN-15, Methyl Derivative. Peak Numbers are Identified in Table 2.

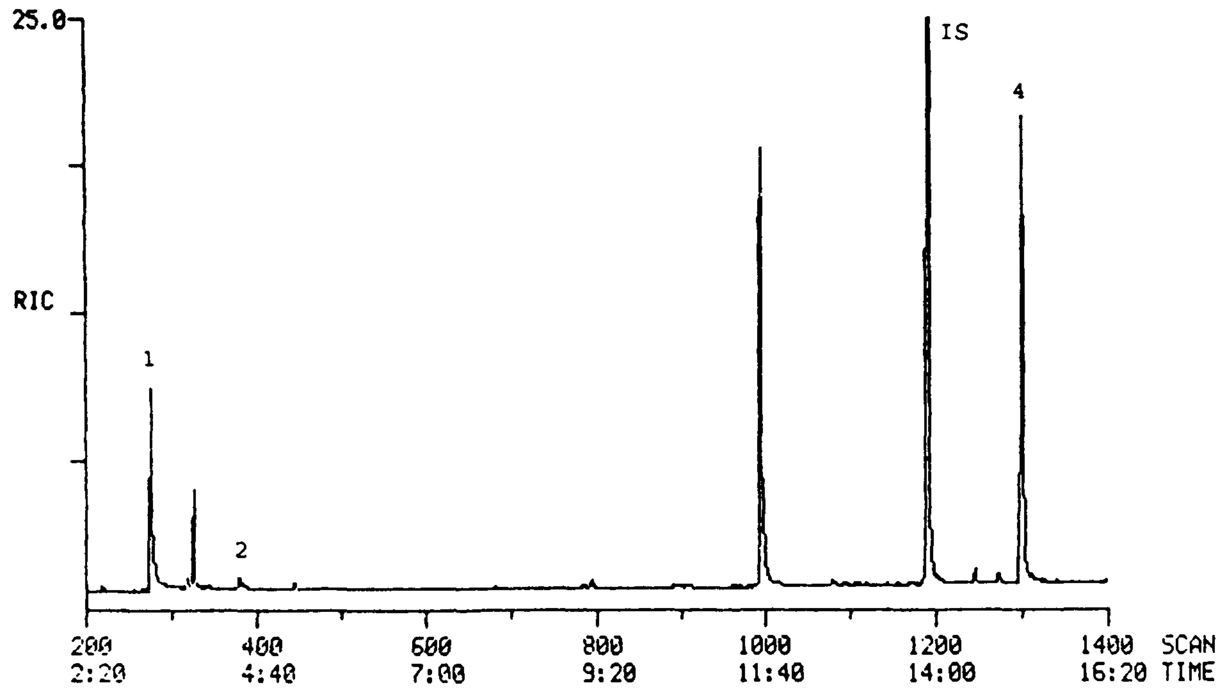


Figure F-20. GC/MS/CI/SIM (Method GC3/MS3) Chromatogram of Water Extract SN-15C* of Soil Sample SN-15, Methyl Derivative. Peak Numbers are Identified in Table 2.

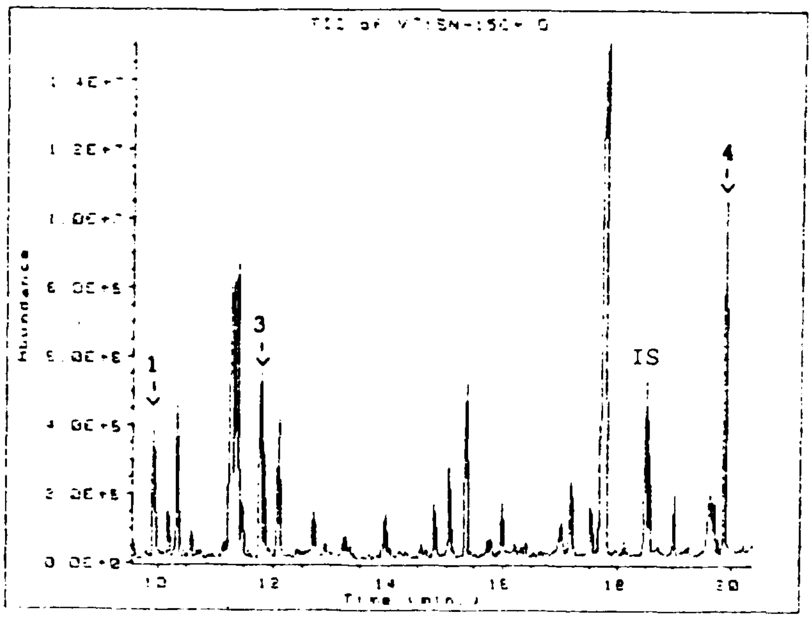


Figure F-21. GC/MSD/EI (Method GC1/MS1) Chromatogram of Water Extract SN-15C* of Soil Sample SN-15, TMS Derivative. Peak Numbers are Identified in Table 2.

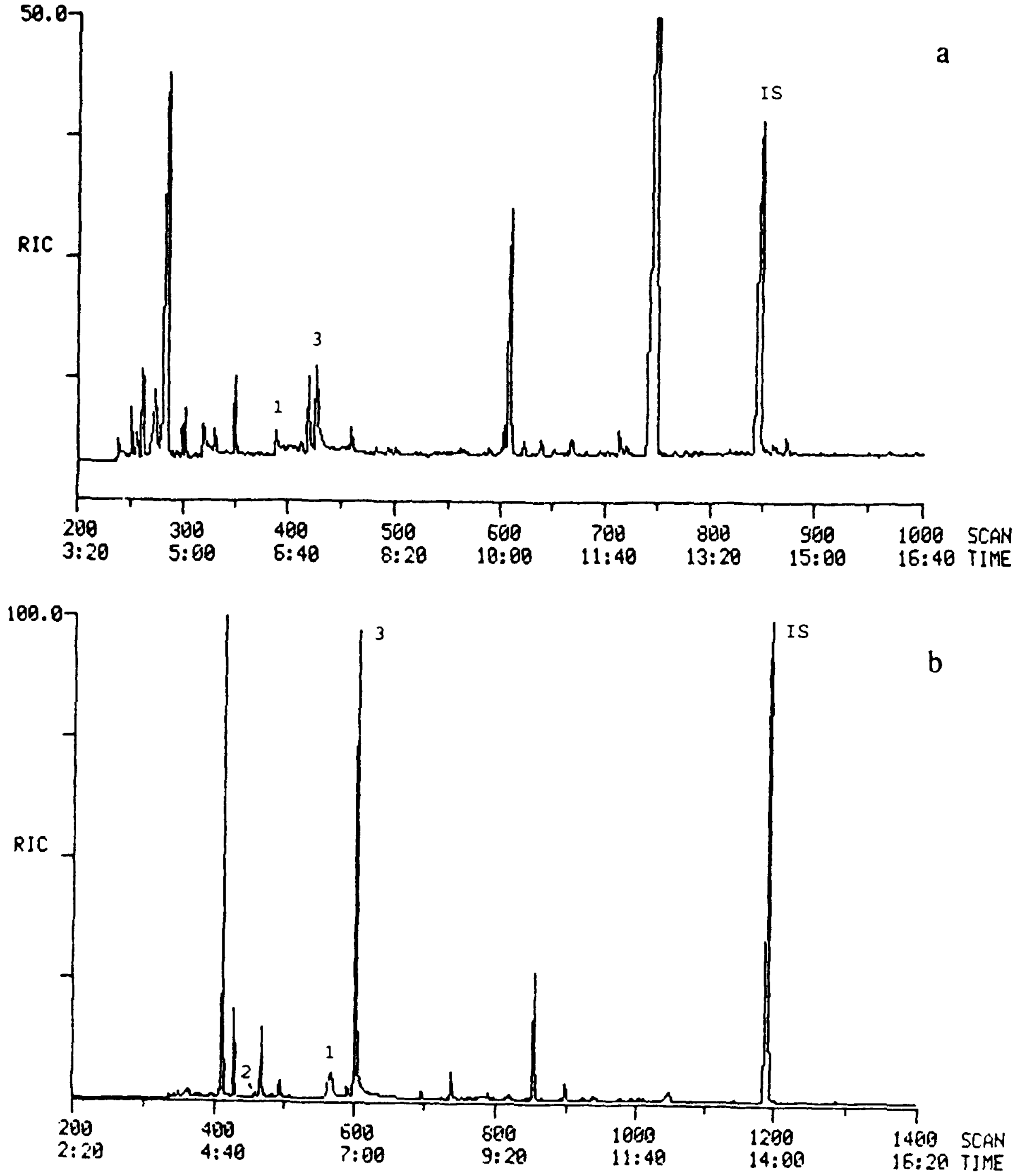


Figure F-22. GC/MS/CI (Method GC3/MS3) a) Full Scan and b) SIM Chromatograms of Water Extract SN-15C* of Soil Sample SN-15, TMS Derivative. Peak Numbers are Identified in Table 2.

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APPENDIX G

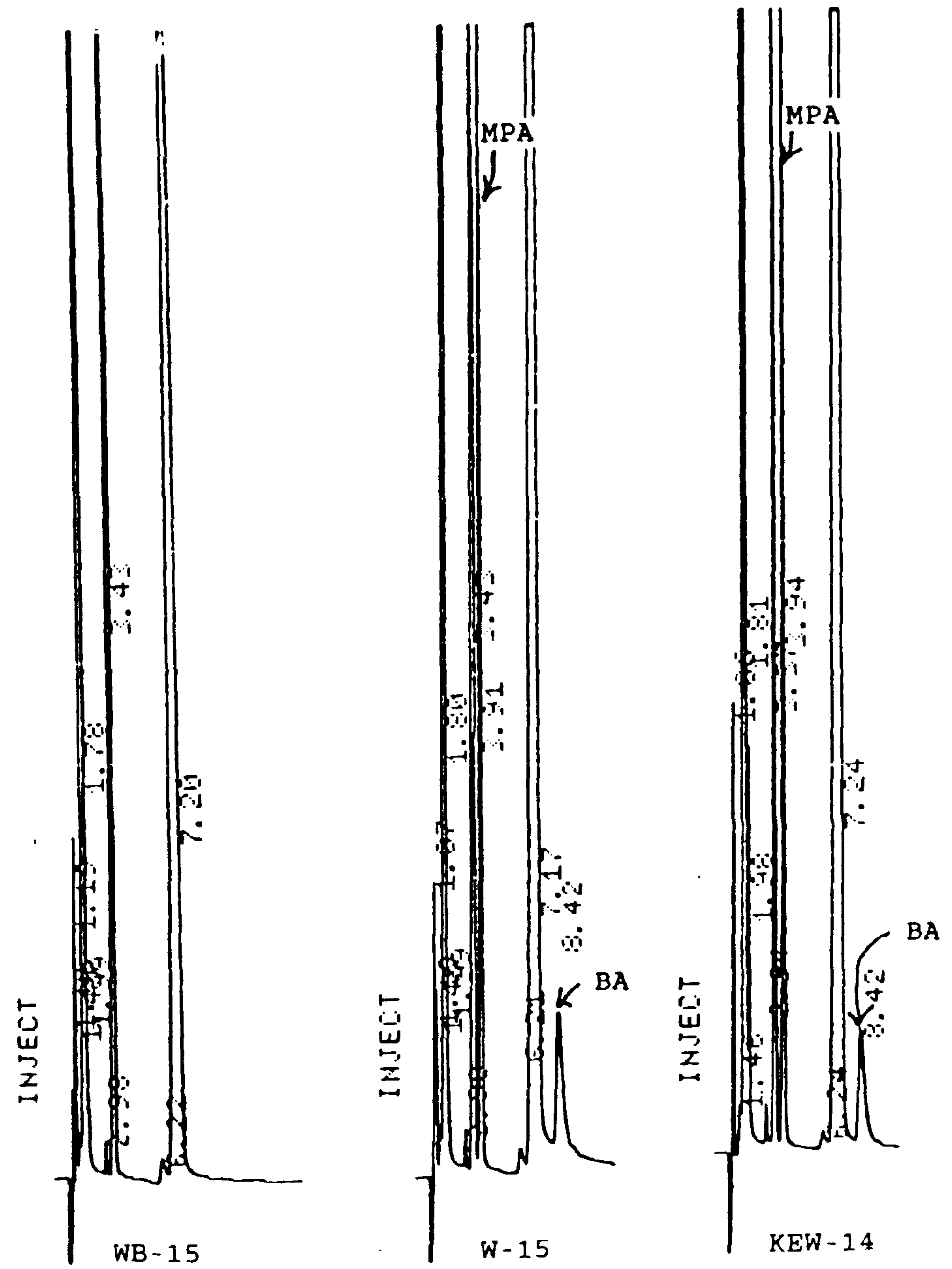
ION CHROMATOGRAPHY METHODS AND CHROMATOGRAMS

Table G-1. Ion Chromatography Method No 1

Instrument: DX-300 (Microbore)	COLUMN: HP1C-AS4A-SC
Detector: Conductivity(10 μSFS)	Manufacturer: DIONEX
HPLC CONDITIONS	Length: 250 mm
ELuent: 0.75 mM $NaHCO_3$/2.2 mM Na_2CO_3 Buffer	Inner diameter: 2 mm
Eluent flow: 0.5 mL/min	
Injection volume: 25 μL	
Sample type analyzed: Natural waters and aqueous soil extracts for methylphosphonic acid, ethyl methylphosphonic acid, isopropyl methylphosphonic acid, pinacolyl methylphosphonic acid and benzilic acid.	

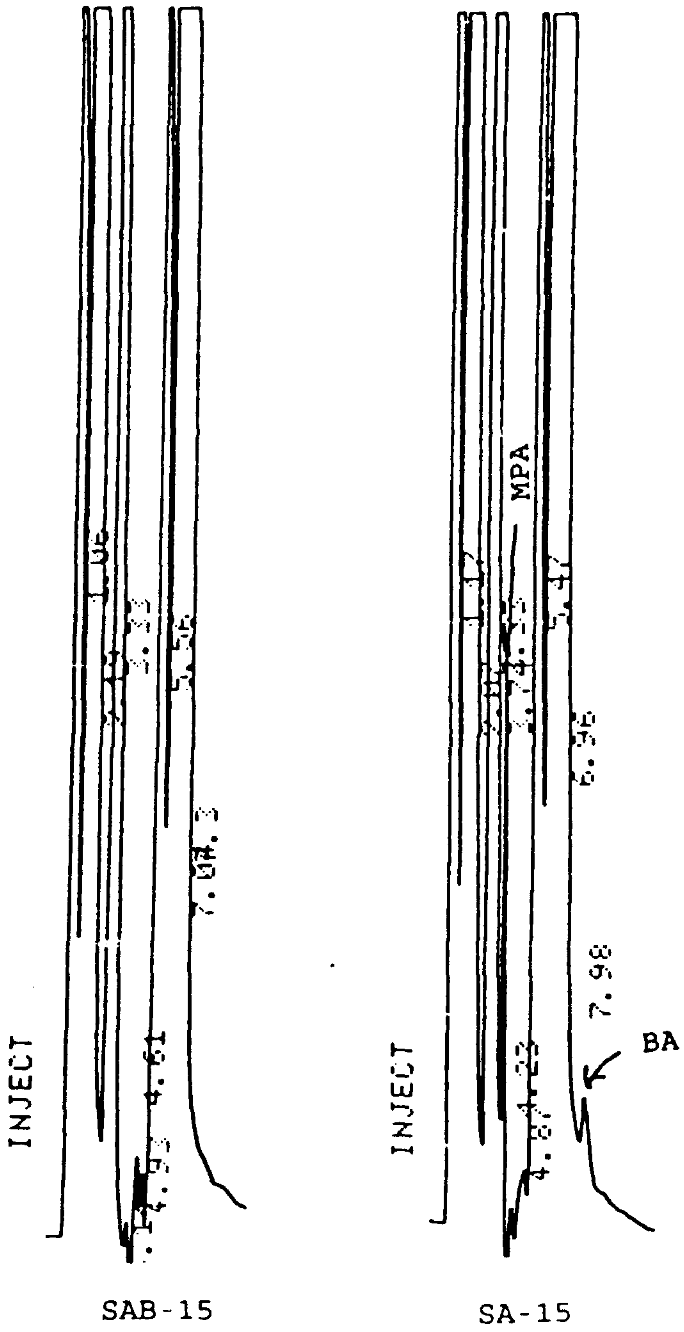
Table G-2. Ion Chromatography Method No 2

Instrument: DX-300 (Microbore)	COLUMN: HP1CE-AS1
Detector: UV (210nM)(0.05 AUFS) PAD(Pt) E1(0.3V)(60mS), E2(1.25V)(60mS), E3(-0.10V)(240mS)	Manufacturer: DIONEX Length: 250 mm
HPLC CONDITIONS	Inner Diameter: 9 mm
ELuent: 100 mM Perchloric Acid	
Eluent flow: 1.0 mL/min	
Injection volume: 200 μL	
Sample type analyzed: Natural waters and aqueous soil extracts for thiodiglycol, thiodiglycol sulfoxide and thiodiglycol sulfone.	



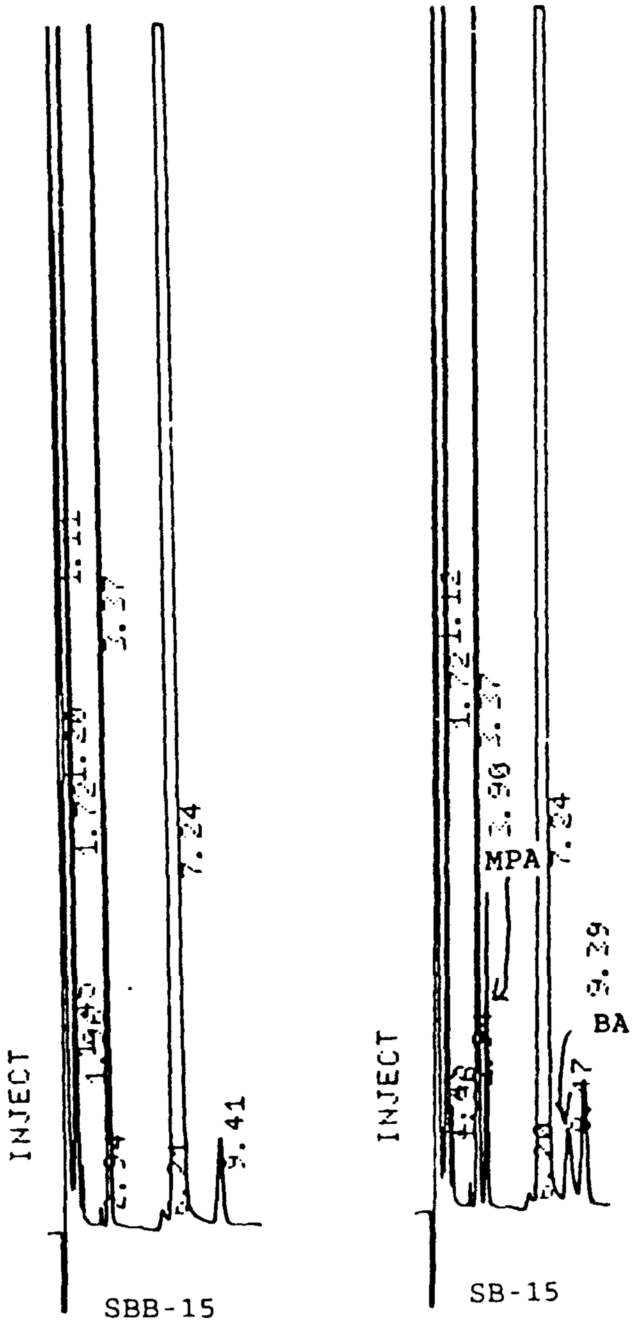
MPA Methylphosphonic Acid
BA Benzilic Acid

Figure G-1. Ion Chromatograms of Water Samples WB-15, W-15 and KEW-14 (Method LC1)



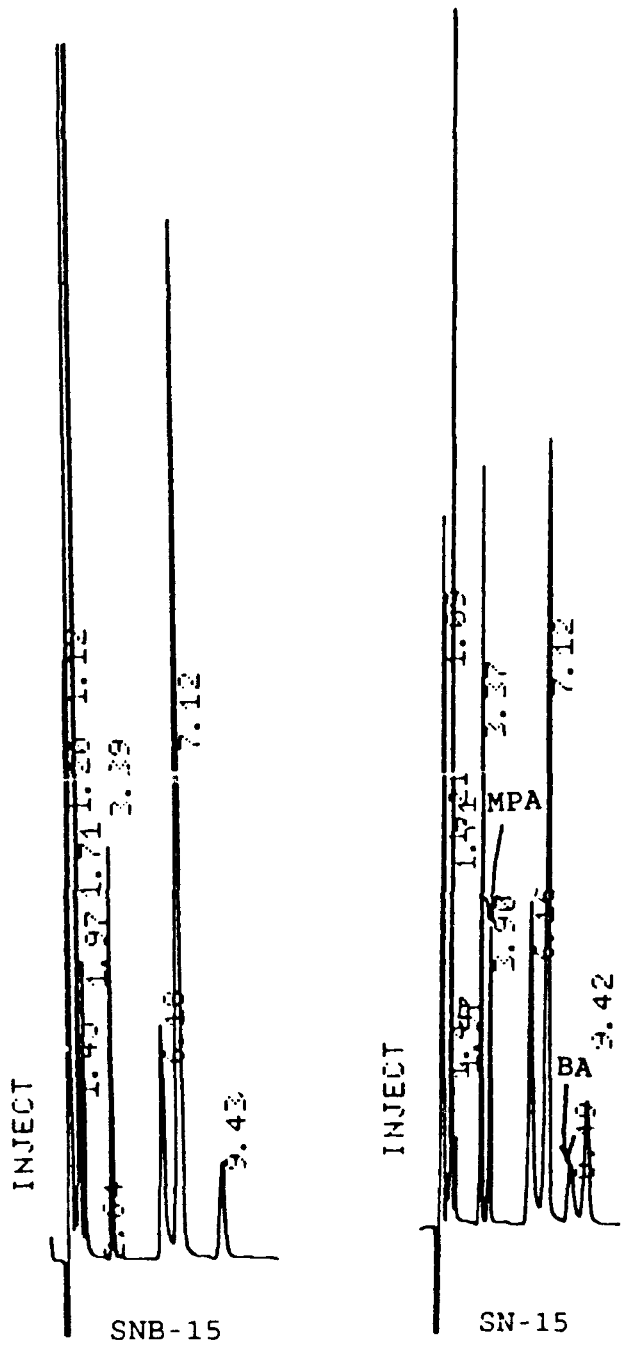
MPA Methylphosphonic Acid
BA Benzilic Acid

Figure G-2. Ion Chromatograms of Water Extracts of Soil Samples SAB-15 and SA-15 (Method LC1)



MPA Methylphosphonic Acid
BA Benzilic Acid

Figure G-3. Ion Chromatograms of Water Extracts of Soil Samples SBB-15 and SB-15 (Method LC1)



MPA Methylphosphonic Acid
BA Benzilic Acid

Figure G-4. Ion Chromatograms of Water Extracts of Soil Samples SNB-15 and SN-15 (Method LC1)

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APPENDIX H

QA/QC PLAN

Document Title: Laboratory Quality Control Plan for International Treaty Verification Round Robin Exercises

Document Designation: Research and Technology
QAPjP CD 03/93/001

Organization: Science Department
Research and Technology Directorate
Edgewood Research, Development and Engineering Center
Attn: SCBRD-RT
Aberdeen Proving Ground, MD 21010-5423

Responsible Official: Director of Research and Technology
Telephone: (410) 671-3250

QA Official: Mr. Dennis W. Johnson
Quality Assurance Coordinator
Research and Technology Directorate
Telephone: (410) 671-4881

Plan Coverage: Analysis of International Treaty Verification Round Robin Samples

Approval:

1. Name: Dennis W. Johnson
   Title: Quality Assurance Coordinator for Research and Technology Directorate
   Signature: [signature] Date: 3 Mar 93

2. Name:
   Title: Director of Research and Technology
   Signature: [signature] Date: 8 March 93

1.0 Project Description

The objective of the international treaty verification round robin exercises is to unambiguously identify CW agents, their degradation products, and other scheduled compounds in complex matrices. The purpose of the program is to evaluate existing recommended procedures and to further develop analytical procedures used to verify treaty compliance with respect to use, storage and production of chemical weapons. Analytical techniques used in this program include gas chromatography/mass spectrometry (GC/MS) using both electron ionization (EI) and chemical ionization (CI), gas chromatography with flame ionization detection and flame photometric detection (GC/FID/FPD), nuclear magnetic resonance (NMR) spectroscopy, high pressure liquid chromatography (HPLC)/Ion Chromatography (IC), and gas chromatography/Fourier transform infrared (GC/FTIR) spectrometry.

2.0 Organization and Responsibilities of Personnel

The Director of Research and Technology has the overall responsibility and authority for managing the QA program in accordance with QA policy. The Director assures the QA policy is understood, implemented and maintained and that the scientific staff has the facilities, equipment and training to perform the mission.

The Quality Assurance Coordinator for Research and Technology serves as the proponent for QA and is available to all Research and Technology Directorate personnel for advice. He is responsible for implementing and maintaining the Directorate Quality Assurance Program Plan (QAPP) and the Laboratory Quality Control Plan.

Office Chiefs in the Science Department and the Chemistry Department assure that operations under their purview are executed in compliance with QA guidelines and policies.

The Team Leader of the Analytical Methodology Team within the Chemistry Department is responsible for the overall technical conduct of the program, including interpretation, documentation and reporting of results and serves as the point of contact for the overall conduct of the investigation. The Team Leader is responsible for checking the consistency of the results obtained for each technique and accepting the final report. The Team Leader may ask for additional experiments to clarify ambiguities between results obtained by different methods to eliminate the reporting of irrelevant data. He is responsible for evaluating the QA QC status of the program and ensures that any necessary corrective actions are taken.

Investigators on the Analytical Methodology Team and the Sample Analysis Team bear the responsibity for the quality of their work. They are responsible for providing QC outputs as required.

following good laboratory practices and manufacturer's specifications, assuring performance and documentation of required maintenance, and taking necessary corrective actions and reporting problems and corrective actions to the Team Leader.

The team leader for this program is Dennis Rohrbaugh (x2366). J Michael Lochner (x2730) serves as sample custodian and does sample preparation. Stephen Pleva and Janet Reeder (x2818) are responsible for electron ionization GC/MS analysis and derivatization procedures. Dennis Rohrbaugh is responsible for chemical ionization GC/MS analysis. Linda Szafraniec and William Beaudry (x3863) are responsible for NMR analysis. Paul Bossle (2794) is responsible for HPLC/IC analysis. Michael Ellzy, L. Gail Janes and Thomas Rosso (x2790) are responsible for GC/FID, GC/FPD, GC/Ion Trap and GC/FTIR analysis.

3.0 QA Objectives for Measurement of Data

The primary focus of this program is to identify unambiguously CW scheduled compounds and their degradation products which may provide evidence for treaty compliance or non-compliance. For this reason, the most important aspect of the program is the interpretation of the chromatographic and spectral data. All spectral data should be compared to that of authentic reference compounds, if possible. If authentic reference compounds are not available, then comparison to data in a spectral library obtained from authentic compounds is acceptable. In the absence of reference material or spectra, tentative identifications may be reported which draw on the overall expertise of the analysts. Compound identification is validated when identified by two separate spectroscopic techniques. Validation also occurs through the use of control samples, reagent blanks and spiked samples. Quantitation is less important than compound identification for this program and will be done by comparison of sample data to that obtained on a standard calibration curve using linear regression analysis or with the use of an internal standard.

4.0 Sampling Procedures

Sampling is not required for this program. Round robin samples are prepared by the sample preparation laboratory to simulate as closely as possible recommended operating procedures listed for sampling of soil (SC5, page 147) and aqueous (SC6, page 149) samples in the third bluebook in the round robin series, "International Inter-laboratory Comparison (Round-Robin) Test for the Verification of Chemical Disarmament F.3. Testing of Procedures on Simulated Military Facility Samples", The Ministry of Foreign Affairs of Finland, 1992, hereafter referred to as Round-Robin Bluebook F.3. For example, methylene chloride is added to the samples for sample preservation and C18 extraction cartridges are included to simulate extraction in the field.

5.0 Sample Preservation

Upon receipt in the laboratory, samples will be documented and the condition of the shipping container and each sample noted. To protect samples from degradation, evaporation, contamination and theft, the samples will be stored in an uncontaminated, lockable refrigerator suitable for storage of flammables at 4°C or lower until removed for sample preparation or analysis.

6.0 Sample Custody

Receipt of the samples will be by the person assuming responsibility for the custody of the samples or his designated alternate. Samples will be stored in a secured freezer. Only authorized personnel will have access to the secured area. The responsible person will keep a custody log to identify individual analysts who receive and return samples for analysis if deemed necessary. Samples will be stored in a high security exclusion area, building E3300. Samples will be analyzed within E3300 and will be in the possession of the analysts or in a secured room at all times. Any transfer of materials outside building E3300 will be documented by form 4137. All samples should be returned for secure, refrigerated storage at the end of the day, unless sample analysis continues overnight (i.e., overnight NMR experiments. The sample custodian for this program is J. Michael Lochner.

7.0 Sample Preparation

Quality assurance and quality control in sample preparation are provided by following the guidelines recommended in section SP10, Quality Assurance and Quality Control in Sample Preparation, in Round-Robin Bluebook F.3., page 173 as follows:

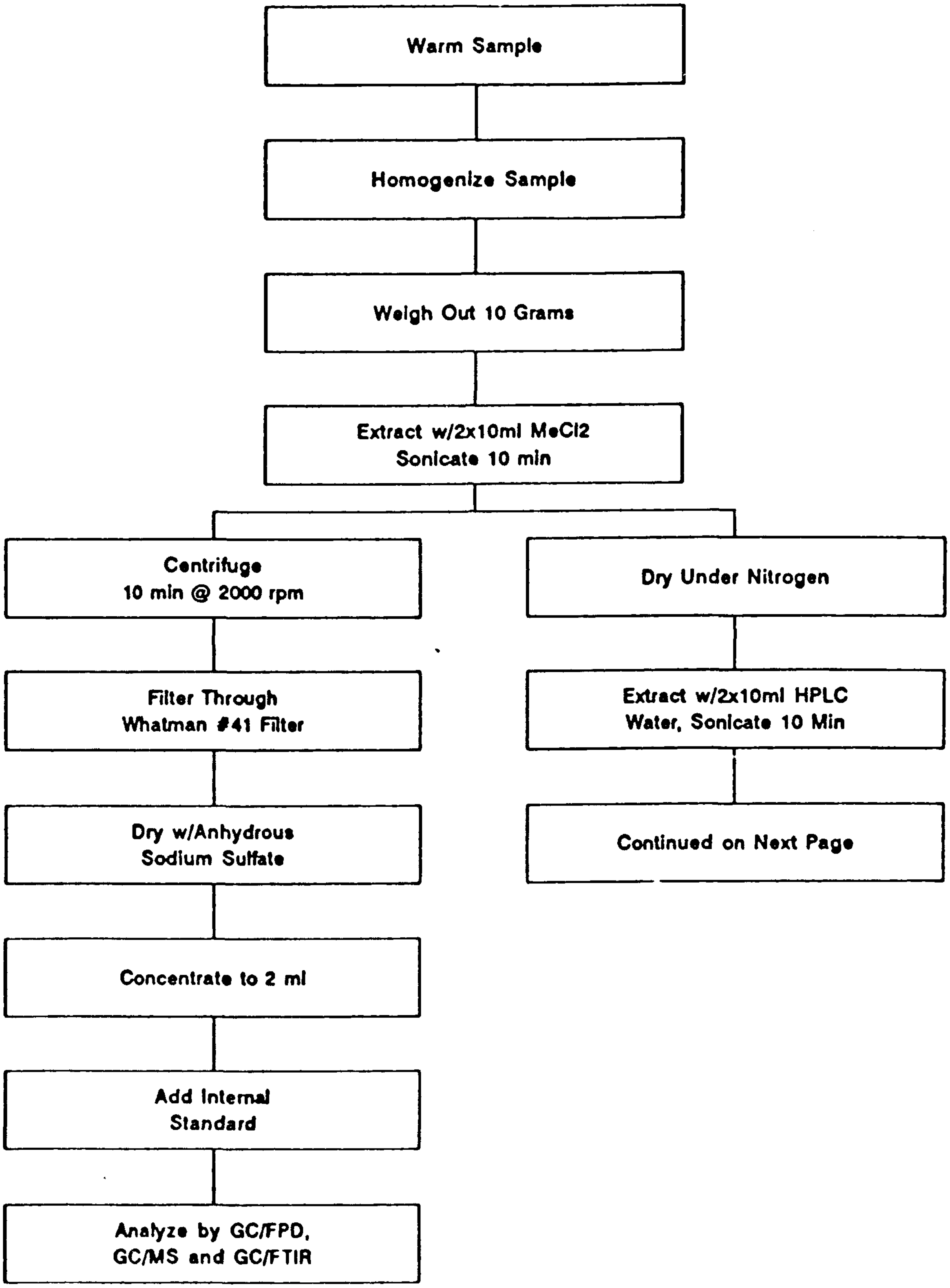
(1) Recommended operating procedures listed in the above blue book reference will be followed.

(2) The purity of solvents and reagents will be checked before sample preparation with the same methods used for analysis.

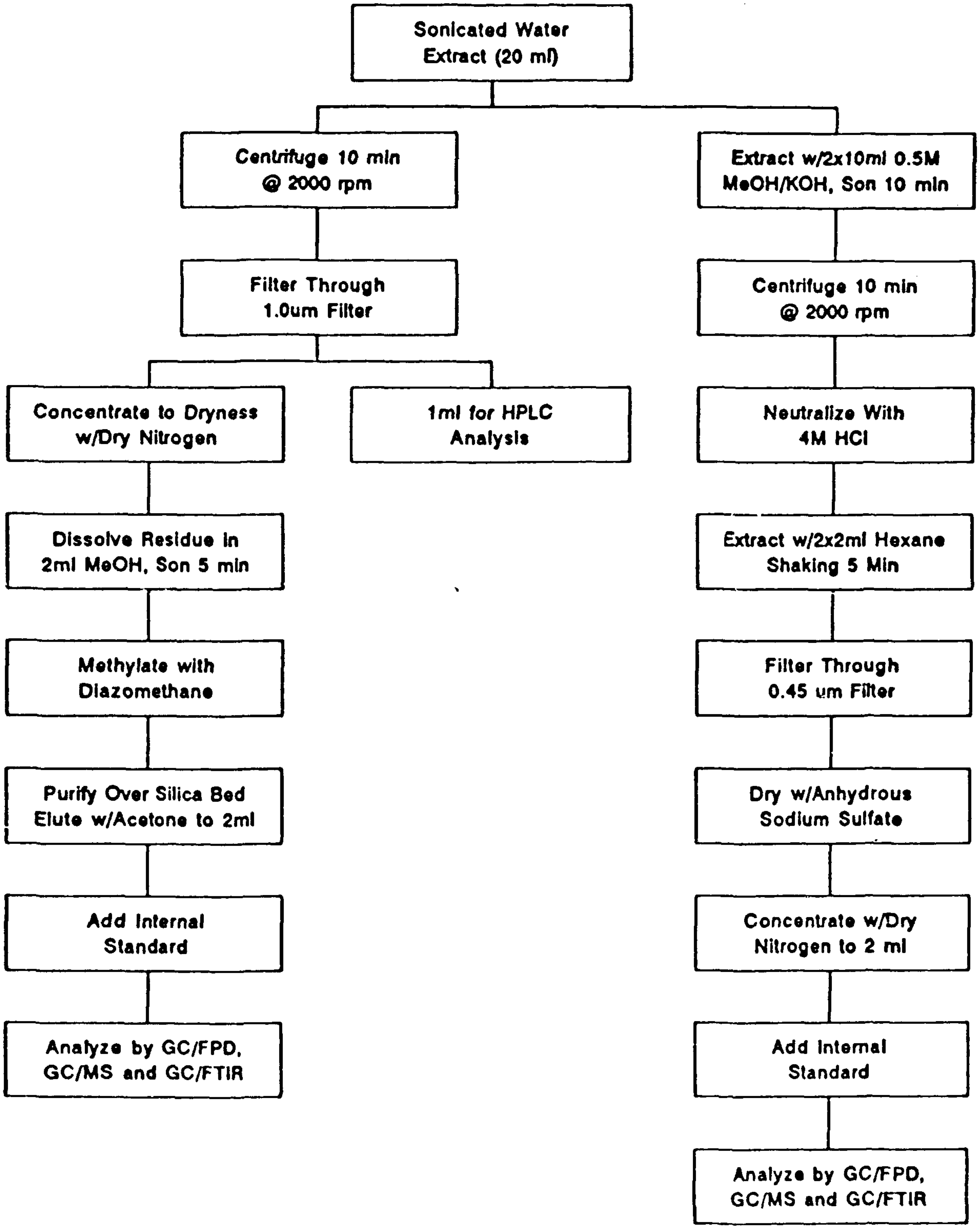
(3) A test run of the whole sample preparation procedure will be run without samples using the same solvents, same reagents and similar glassware to investigate the background generated by the procedure.

(4) At least two parallel samples will be run, if time permits. In this study, two parallel extractions will be done, one in deuteratd solvents for NMR analysis and one in non-deuterated solvents for all other methods of analysis.

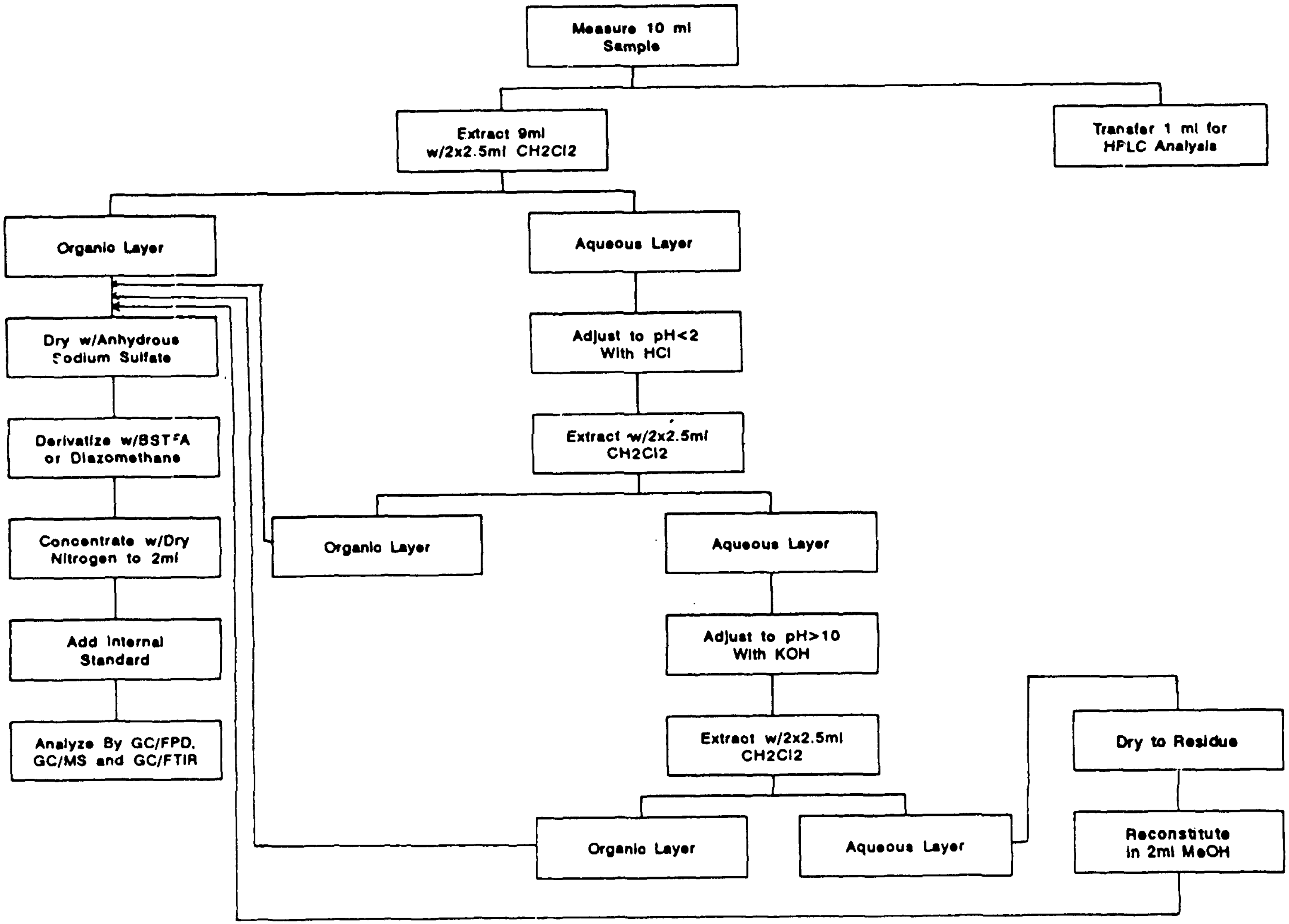
SOIL SAMPLE PREPARATION



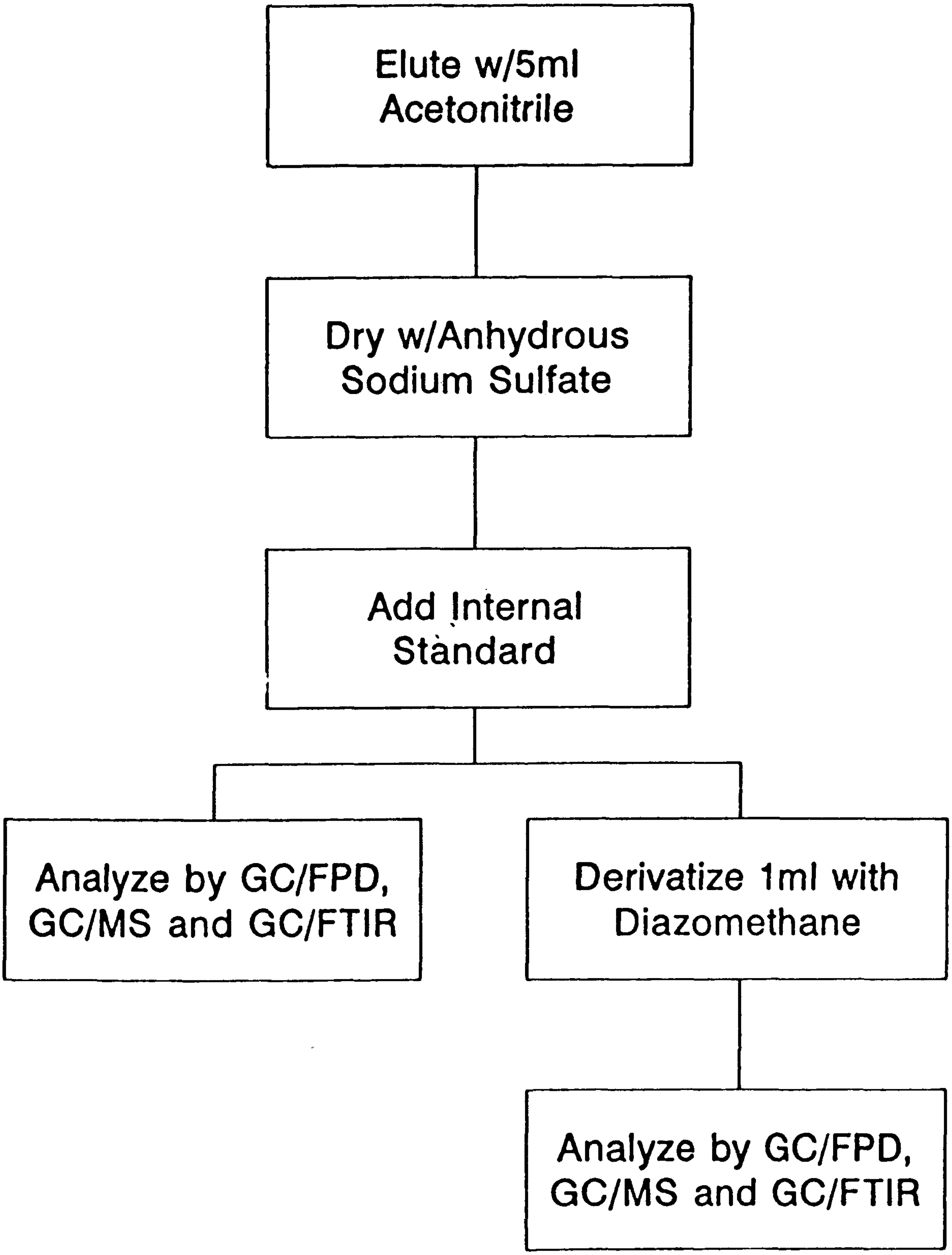
SOIL SAMPLE PREPARATION (CONT'D)



AQUEOUS LIQUID SAMPLE PREPARATION



C18 CARTRIDGE PREPARATION



(5) If time permits, the recovery of the sample preparation method will be tested by spiking the blank sample with a known amount of the compounds of interest and repeating the sample preparation (this assumes that a clearly marked blank sample of the same matrix has been delivered with the sample.

(6) Separate glassware will be used for spiked samples and blank tests to avoid cross-contamination.

7.1 Preparation of soil samples

Soil samples will be prepared according to recommended operating procedure SP4 of Round-Robin bluebook F.3. (page 161). This scheme is shown on the attached flow chart. In addition to the recommended procedure, a parallel extraction will be done using the deuterated solvents $CDCl_3$ and D_2O for NMR analysis using the modified scheme shown on the attached flow chart.

7.2 Preparation of aqueous samples

Aqueous samples will be prepared according to recommended operating procedure SP7 of Round-Robin bluebook F.3. (page 167). In addition to the recommended procedure, parallel extraction will be done using the deuterated solvents $CDCl_3$ and D_2O for NMR analysis using the modified scheme shown on the attached flow chart.

7.3 Preparation of C18 cartridges

C18 extraction cartridges will be prepared by elution with acetonitrile or other suitable solvent, drying with sodium sulfate and derivatization with diazomethane as shown in the scheme below.

8.0 Calibration Procedures

Instrument calibrations are performed as described below or within the analytical methods. In general, calibrations are performed using standards supplied by instrument manufacturers and in accordance with manufacturer's specifications. Test mixtures are then run to check instrument performance and sensitivity. Once the target analytes have been identified, these test mixtures can be modified to include compounds whose structures are similar to those of the analytes of interest.

8.1 GC/MS

Daily calibration of GC/MS instrumentation is performed using the calibration standard perfluorotributylamine (PFTBA or (FC43) provided in sealed ampoules by the instrument manufacturer. In this step the mass range is calibrated and the instrument is tuned to measure correctly the relative abundances of the calibration gas. Calibration can be performed using either the software driven autotune program or manually as long as the final tune is in accordance with the manufacturer's specifications. The performance of the instrument in then checked with a multipurpose test solution with concentrations close to the detection limit of the instrument. As a further test of instrument performance, a system blank is run with the solvent used to prepare the samples. PFTBA calibration is done daily. Test solutions and system blanks are run with each new set of samples. Additional information is provided in the methods section.

8.2 Gas Chromatography

Detector-specific test mixtures are used to monitor GC sensitivity, column performance, and retention time stability. These mixtures are run with each new set of samples and as a minimum every 20th injection. Specific test mixtures for each detector are outlined in Chemistry Department Method No. 002.00. System performance is also checked by use of blanks to avoid false positive identifications through memory effects from either the syringe or instrumentation. Calibration for quantitation is accomplished through the use of internal standards or a multipoint calibration curve which brackets the concentrations of the analytes of interest. A house checkpoint sample is run daily to verify that the calibration curve is still valid. If the house checkpoint sample falls outside a +/- 10% range, the instrument is recalibrated or corrective actions are taken.

8.3 NMR

The NMR spectrometer systems are under service contract with the manufacturer (Varian). Calibrations of pulse widths, decoupler field strengths, and other parameters are done on a routine basis and whenever repairs are performed. All calibrations are performed using the sealed standard samples provided by the manufacturer and in accordance with the manufacturer's specifications as outlined in Varian Instrument Division Publication No. 87-178115 Rev. A1285, "VXR Series NMR Spectrometer System Acceptance Test Procedure."

8.4 HPLC/Ion Chromatography

Calibration of the HPLC is done in a manner similar to the GC procedure. System performance is monitored with a test mix Blanks are run before each set of samples to eliminate false positives. Linear regression is used to determine the minimum detection limit and to plot a calibration curve from a stock solution and its set of serial dilutions. A working standard is used daily as a house checkpoint.

8.5 FTIR

The optics of the IR are aligned and calibrated to produce the best signal according to the manufacturer's instructions using the test mix provided by the manufacturer (i.e. polystyrene). A tune macro is run every day to show the center-burst from the interferogram and to correct the gain or mirror velocity. A 100% line will ratio a background scan and the result should be a straight line +/- 0.05%. Along with the 100% line, a signal-to-noise macro is run every day with an acceptable ratio of above 600:1.

8.6 Balances

Balances used to prepare quantitation standards receive annual calibration performed and documented by the U.S. Army Test, Measurement and Diagnostic Equipment Support Center. The DA Label 80, affixed when calibration occurs, specifies the next calibration date and assures that the balance is in the balance recall system. NIST traceable weights which bracket expected sample weights are used as balance check standards before daily useage.

9.0 Reference Standards

Reference standards for spectral comparison and quantitation are required. The standard agents GA, GB, GD, VX, HD and L are obtained from the Chemical Agent Standard Analytical Reference Material (CASARM) program. This program maintains high purity neat and dilute agent samples in sealed ampoules stored at low temperatures. The purity of the standards is carefully monitored periodically as requested by the CASARM committee and the ERDEC Compliance Management Directorate. Other agents may be made available by synthesis in the laboratory. Schedule 2 and schedule 3 compounds may be available commercially.

10.0 Analytical Procedures

Analytical techniques used in this program include GC/MS, GC (FID, FPD), multinuclear NMR, HPLC (Ion Chromatography) and GC-FTIR. Specific analytical procedures for each, including instrument calibration, reference materials, apparatus required, procedures, and treatment of data are described in the Methods section and elsewhere in this document. Procedures were selected to follow as closely as possible the following sections of Round Robin Bluebook F.3.:

GC3	GC/FID/FPD	(pages 175-182)
LC3	HPLC/IC	(pages 187-188)
MS1	GC/MS	(pages 189-204)
NMR1	NMR	(pages 209-226)
IR1	GC/FTIR	(pages 227-232)

GC/MS is the primary method used for compound identification. GC/FPD is used as an important screening tool to selectively detect compounds which contain phosphorus and sulfur. HPLC/IC is used to identify and quantitate ionic species present in the water fractions. NMR and GC/FTIR are used primarily as confirmatory techniques but also have a role to play in compound identification.

10.1 Chromatographic Order of Analysis

The recommended order of analysis for all chromatographic techniques performed, to eliminate the possibility of false positives, is the following: 1) test solution, 2) solvent blank, 3) sample. The test solution is used to verify that the instrument is working properly. The solvent blank is run to verify there is no sample carryover in the system resulting from syringe, injection port or column memory effects or from a contaminated solvent used to clean the syringe. If memory effects are observed, analysis will be discontinued until the source is found and corrected. Test solutions and blanks should be run at the beginning of each day, at the start of each new series of samples, and as a minimum every twentieth sample. Solvent blanks should also be run between samples if carryover is suspected.

10.2 Quantitation

Internal standard (IS) is the preferred method of quantitation. Primary quantitation will be done by GC/MS/EI and/or GC/MS/CI using selected ion monitoring (SIM) for low level analytes. Full scan can be used for higher concentrations when no interference is observed. The IS will be added after sample preparation and before analysis. The approach is to screen a representative prepared sample of a series before addition of the IS by GC/FID/FPD and/or GC/MS and to select as an IS a compound from the GC/MS test mixture that does not interfere with any peaks in the chromatogram. The IS will then be added to all prepared samples in the series. The concentration will be based on the analyte concentrations estimated from the sreening. As an alternative, a deuterated compound may be used as the IS. The same samples can also be quantitated by GC/FID/FPD or GC/FTIR to obtain additional comparison of data, but is not required. GC response factors will be determined when authentic samples of the analytes are obtained. Analyte concentrations are calculated using the following equation:

$$C_x = F(A_x/A_{st}) \times C_{st}$$

where

F = Response Factor
A_x = Peak Area of Analyte
A_{st} = Peak Area of Standard
C_x = Concentration of Analyte
C_{st} = Concentration of Standard

Compounds detected by HPLC are quantitated with a calibration curve and linear regression analysis using established procedures. NMR is not used for quantitation.

11.0 Data Reduction, Validation, and Reporting

Most data reduction, including the capture, processing and storing of data, is handled by documented computer software programs. The instrument calibration procedures performed are a form of data system validation. Project data must be traceable and retrievable. Raw data not stored in a data system is maintained in a file for future reference. Procedures, calculations and results are maintained in a laboratory notebook or on data sheets. Interpretations and quantitation calculations are checked by a second investigator. Correlation of the results from the different methods is the most important form of validation. Identification by at least two different spectrometric techniques constitutes unambiguous identification. Comparison to data obtained from authentic samples is required for unambiguous identification. Data is also validated through the use of blanks and spike recoveries.

A preliminary report listing results is sent to the coordinating laboratory within one month of the start date of the round robin. A more extensive report listing analytical procedures, QA/QC procedures and findings is due two weeks later.

12.0 Internal QC Checks

Since most procedures outlined in this program are carried out by more than one investigator, internal QC checks are performed by a second investigator who has expertise in that particular area who reviews the data. Quality control samples, other than the reference materials and calibration standards delineated above, are not normally required for this program.

13.0 Preventive Maintenance

Instruments shall be maintained to assure that data generated is acceptable. Instruments are calibrated according to documented procedures. A log book will be maintained for each instrument documenting all routine maintenance and repairs, including the date, the service condition and the repairs required. The log book will be maintained as specified in Research Directorate SOP QAU-5, entitled Instrument Log Books. Any equipment item that gives suspect results or has been shown by verification testing to be defective shall be taken out of service until corrective actions can be taken.

13.1 GC/MS

Maintenance of all GC/MS instrumentation is performed under service contracts with the manufacturers. Oil changes, source cleaning, air filter changes, septa replacement and other routine maintenance is performed by in-house personnel. Major repairs are performed by the manufacturer. The service contract also calls for two preventive maintenance visits a year by the manufacturer.

13.2 GC

GC preventive maintenance is done by in-house personnel. Septa, injection port liners, columns, ferrules, FID jets, etc. are replaced as required. Maintenance contracts are in place with the instrument manufacturers for more serious repairs.

13.3 NMR

Maintenance of the NMR spectrometers is performed under service contract with the manufacturer. The only service performed by in-house personnel is weekly replacement of liquid nitrogen.

13.4 HPLC/IC

Maintenance of the HPLC is performed via a comprehensive service contract with the manufacturer. Injection needles, columns, electrodes (working and reference) and fittings and tubing are available within the laboratory for use on an as required basis.

13.5 FTIR

Maintenance of the GC-FTIR is performed under a comprehensive service contract with the instrument manufacturer.

14.0 Internal Audits

Internal audits are performed announced and/or unannounced at the discretion of the Research and Technology Directorate Quality Assurance Coordinator to assure the Director that the requirements of the QAPP and the QC plan are being followed. Audits provide an objective evaluation of quality related practices, procedures and activities to include the review of documents and records. Audits will be conducted and documented according to Research Directorate SOP QAU-2, Procedure for Audits by the Quality Assurance Unit. Records of all audits will be maintained by the Quality Assurance Unit (QAU) for a period of 5 years.

15.0 Corrective Actions

Research and Technology Directorate will use timely corrective action to minimize the generation and reporting of erroneous data. Instrument failure and the exceeding of calibration limits are the primary problems which require corrective action. Corrective action shall be documented in the laboratory notebook or maintenance log by citing the problem, the corrective action taken, and documenting that acceptable data generation has resumed.

16.0 Applicable Safety Regulations and Standard Operating Procedures

(1) AMC-R-100 Safety Manual (1 Aug 1985)

(2) AMC-R-385-131 Safety Regulation for Chemical Agents H, HD, HT, GB, and VX (9 Oct 1987).

(3) CRDEC-R-385-1 Chemical, Explosive and Occupationl Safety and Health Program (15 Aug 1986).

(4) CRDEC-R-385-10 Certification Program for Personnel Involved in Hazardous Operations (1 Jun 1986).

(5) CRDEC-R-385-12 Hazard Communication Program (15 Jul 1987).

(6) CRDEC-R-385-14 Chemical Safety Program (1 Aug 1990).

(7) GOP 8-0-90-0000 General Operating Procedure for Safety Surety Operations in Research Directorate (6 Jul 1990).

(8) SOP 8-3-92-1000 The NMR Experiment (18 Sep 1992).

(9) SOP 8-0-90-0005 Handling and Sampling of Chemical Agents (3 Dec 1990).

(10) CR8-OSP 0005 Filling of NMR Tubes (11 Feb 1993).

17.0 Analytical Methods

Chemistry Department Method 001.00. Gas Chromatography/Mass Spectrometry (GC/MS) Procedures for the International Interlaboratory Treaty Verification Round Robin Exercises.

Chemistry Department Method 002.00. Gas Chromatography (GC) procedures for the International Interlaboratory Treaty Verification Round Robin Exercises.

Chemistry Department Method 003.00. Nuclear Magnetic Resonance (NMR) Spectroscopy Procedures for the International Interlaboratory Treaty Verification Round Robin Exercises.

Chemistry Department Method 004.00. High Performance Liquid Chromatography (HPLC) Procedures for the International Interlaboratory Treaty Verification Round Robin Exercises.

Chemistry Department Method 005.00. Fourier Transform Infrared (FTIR) Procedures for the International Interlaboratory Treaty Verification Round Robin Exercises.

GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) PROCEDURES FOR THE INTERNATIONAL INTERLABORATORY TREATY VERIFICATION ROUND ROBIN EXERCISES

1. DATE: February 1993

2. PREVIOUS REVISIONS: None

3. OFFICE OF CONTACT.

Analytical Methodology Team, Chemistry Department
Research and Technology Directorate, ERDEC
SCBRD-RTS
POC: Dennis Rohrbaugh (2366), Stephen Pleva (2818),
Michael Ellzy or Thomas Rosso (2790)

4. APPLICATION: This method applies to the use of gas chromatography/mass spectrometry (GC/MS) for the detection and identification of treaty-related CW scheduled compounds in complex matrices. The method applies to volatile compounds that are extractable into organic solvents. Both electron ionization (EI) and chemical ionization (CI) are applied and serve as complimentary techniques. Selected ion monitoring (SIM), the use of selected ions only, is used to increase sensitivity and lower the detection limit.

4.1 CONCENTRATION RANGE: Detection Limit to Neat

4.2 DETECTION LIMITS: Detection limits are instrument specific and compound specific. In general, detection limits are about 1-10 ug/ml using full scan detection and 0.02-0.05 ug/ml using SIM with the GC/MS instrumentation used in this study. Detection limits are 0.1 ug/ml (full scan) and 0.01 ug/ml (SIM) for the GC/ion trap.

4.3 INTERFERENCES: Any compound which coelutes with the analytes of interest.

4.4 ANALYSIS RATE: One sample per 30 minutes.

4.5 VALIDATION: Compound identifications are validated by comparison of GC retention times and mass spectra to those of authentic samples either by direct comparison or by comparison to spectra in a data base library. Identification by both EI and CI methods is a form of validation. Comparison to authentic samples and confirmation by at least 2 separate spectrometric techniques constitutes unambiguous identification.

5.0 SCIENTIFIC BASIS: Analytes of interest are separated from solvent and matrix background by elution on a capillary gas chromatographic column. In electron ionization, components are bombarded with electrons as they enter the mass spectrometer ion source. Resulting positive-charged fragment ions are detected separately by an electron multiplier after passing through a quadrupole mass filter which discriminates according to mass/charge ratio. The resulting mass spectrum is characteristic of a particular compound. Chemical Ionization is a softer form of ionization where a reagent gas such as methane is ionized in the ion source. The resulting CH_5^+ then chemically reacts with the analyte. The advantage of this technique is that because less energy is involved there is less fragmentation and therefore a greater probability of seeing the molecular ion and thus determining the molecular weight of the compound. This technique is particularly useful for analyzing large molecules which readily undergo extensive fragmentation under EI conditions, like VX and VX degradation products. Ammonia chemical ionization is particularly useful as a specific ionization technique for organophosphorus compounds.

In the ion trap ions are stabilized or destabilized in an electric or magnetic field. Compounds are swept into the filament, with an emission current of 80 uamps. The accelerated electrons cause ionization in the ITD cavity where a storage voltage is set to "trap" all ions of interest. Mass analysis occurs as the amplitude of the RF voltage is increased, while holding the frequency constant, thus causing the ions to destabilize in the z-axis. 50% of the ions travel up and are neutralized and 50% of the ions travel through the bottom end cap and are detected by the electron multiplier. This process is repeated for each scan segment used during analysis.

6. APPARATUS:

6.1 INSTRUMENTATION:

Hewlett-Packard 5870 quadrupole Mass Selective Detector equipped with an HP 5890 GC interfaced with a Tekmar 5010 Liquid Nitrogen Cryofocus @ -120°C. Used for EI only.

Finnigan 5100 quadrupole GC/MS equipped with a Finnigan model 9610 GC. Used almost exclusively for CI.

Finnigan Ion Trap ITD-40 equipped with a Perkin-Elmer 8500 GC. Used for both EI and CI.

6.2 HARDWARE/GLASSWARE: Microliter syringes

6.3 REAGENTS: Utrahigh purity carrier gas (helium) and chemical ionization reagent gases (methane, isobutane or ammonia). Purity must be greater than 99.995%.

6.4 GAS CHROMATOGRAPHIC COLUMNS: Recommended column for this program is a fused silica SE-54 (DB-5), 25 m x 0.2mm i.d., 0.25 um film. DB-1 and DB-1701 also give satisfactory results.

7. STANDARDS:

7.1 CALIBRATION STANDARD: Perfluorotributylamine (PFTBA or FC43) supplied in sealed ampoules by the instrument manufacturer.

7.2 TEST MIXTURE: Recommended test mixture used to monitor instrument performance is as follows:

Test Compound	Concentration (ug/ml)
Trimethyl Phosphate	20
2,6-Dimethylphenol	20
5-Cl-2-Methylaniline	20
Tri-n-butyl Phosphate	20
Dibenzothiophene	20
Methyl Stearate	20

Solvent = Ethyl acetate or Hexane

8. PROCEDURES:

8.1 EI INSTRUMENT CALIBRATION: Mass range and relative ion intensities are calibrated using PFTBA calibration gas supplied in sealed ampoules by the instrument manufacturer. The PFTBA is tuned to maximun sensitivity until the following criteria are met.

m/z 69	100%
m/z 219	30-60%
m/z 414	1.4-4.0%
m/z 502	0.8-4.0%

The resolution of the m/z 69,70 and m/z 502,503 is tuned until a 10% valley and good peak shape are obtained. Isotope ratios are adjusted to match the true values of PFTBA as closely as possible; 70:69 = 1.1, 220:219 = 4.3, 415:414 = 9.0 and 503:502 = 10.1. A variance of 20% is acceptable. The masses are then calibrated using the intrument's automatic calibration program. Instrument performance is also monitored by using the test solution. Signal-to-noise ratio should be >10:1, m/z values should be correct, and deviation of relative ion abundances should be less than +/-20%. Intensity ratios of the isotopic ions should be within specification as outlined in the reference.

8.2 CI INSTRUMENT CALIBRATION: CI tuning is accomplished by following the above EI tuning procedure. Once an acceptable EI tune is obtained, the reagent gas source pressure is adjusted to 0.6 Torr and for methane the resolution is tuned to 10% valley using ions m/z 219, 220 and 414, 415. Intensities are adjusted to meet the following criteria:

m/z 69	< 50%
m/z 219	<100%
m/z 414	100%
m/z 634	> 20%
m/z 652	> 20%

Analysis of the test solution must meet the same criteria as above for EI.

8.3 INSTRUMENT OPERATING PARAMETERS: Instrument operating parameters are somewhat instrument specific. The following are the recommended operating parameters for this program:

GC	Injector Temperature	260°C
	Interface Temperature	260°C
	Septum Flow	5 ml/min
	Carrier Gas Flow	2 ml/min
	Split Ratio	20:1
	Oven Temp Program	40°C (1 min) - 280°C (10 min) at 10°C/min
MS/EI	Electron Energy	70 eV
	Emission Current	0.3 mV
	Ion Source Temperature	120°C
	Scan Range	40-600 amu
	Scan Rate	1 scan/sec
MS/CI	Electron Energy	150 eV
	Emission Current	0.3 mV
	Ion Source Temperature	100°C
	Scan Range	60-600 amu
	Scan Rate	1 scan/sec
	Reagent Gas Source Pressure	0.6 Torr

9.0 TREATMENT OF DATA: GC retention times and mass spectra are compared to those of authentic compounds for both electron and chemical ionization. Results are then correlated with those obtained with other analytical methods in this program. Results are also compared to blank runs.

10.0 REFERENCE: "International Interlaboratory Comparison (Round Robin) Test for the Verification of Chemical Disarmament F.3. Testing of Procedures on Simulated Military Facility Samples", The Ministry of Foreign Affairs of Finland, Helsinki, 1992.

GAS CHROMATOGRAPHY (GC) PROCEDURES FOR THE INTERNATIONAL INTERLABORATORY TREATY VERIFICATION ROUND ROBIN EXERCISES

1. DATE: February 1993

2. PREVIOUS REVISIONS: None

3. OFFICE OF CONTACT:

   Sample Analysis Team, Chemistry Department
   Research and Technology Directorate, ERDEC
   SCBRD-RTS
   POC: L. Gail Janes (3812) or Michael Ellzy (2790)

4. APPLICATION: This method applies to the use of gas chromatography (GC) for the detection and identification of treaty-related CW scheduled compounds in complex matrices. The method applies to volatile compounds that are extractable into organic solvents. Both flame photometric detection (FPD) and flame ionization detection (FID) are used.

4.1 CONCENTRATION RANGE: Detection Limit to Neat

4.2 DETECTION LIMITS: Detection limits are approximately 5 ug/ml for FID, 0.1 ug/ml for FPD/sulfur and 0.01 ug/ml for FPD/phosphorus.

4.3 INTERFERENCES: Any compound which coelutes with the analytes of interest.

4.4 ANALYSIS RATE: One sample per 30 minutes.

4.5 VALIDATION: Compound identifications are validated by comparison of GC retention times to those of authentic samples, preferably using two different types of columns.

5.0 SCIENTIFIC BASIS: Analytes of interest are separated from solvent and matrix background by elution on a capillary gas chromatographic column and detected by one of two detectors. All compounds containing carbon-hydrogen bonds are detected by FID. Phosphorus and sulfur-containing compounds are selectively detected using the FPD. Because most schedule 1 and 2 compounds contain phosphorus and/or sulfur, the FPD is particularly useful in this program because of its high sensitivity and selectivity.

6. APPARATUS:

6.1 INSTRUMENTATION:

GC/FID: Hewlett-Packard 5880A with integrator

GC/FPD (P): Varian 6000 Vista, Vista 402 Data Station

GC/FPD (P and S): Perkin-Elmer 8500, LCI-100 Integrator

GC/FPD (S): Hewlett-Packard 5880A with integrator

6.2 HARDWARE/GLASSWARE: Microliter syringes

6.3 REAGENTS: High Purity Carrier gas (helium or nitrogen), air and hydrogen.

6.4 GAS CHROMATOGRAPHIC COLUMNS: Recommended columns for this program are fused silica SE-54 (DB-5) and DB-1701, 25m x 0.32mm i.d., 0.25 um film or equivalent columns.

7. STANDARDS:

7.1 GC/FPD: Instrument performance is monitored by using a test mix containing 1 ppm dodecanethiol and tributyl phosphate in isooctane. This solution is prepared by dilution of a 20 ppm stock solution provided in sealed ampoules by the instrument manufacturer (Hewlett-Packard).

7.2 GC/FID: Instrument performance is routinely monitored by injection of a solution containing 0.033 wt% each of n-tetradecane, n-pentadecane and n-hexadecane in hexane provided in a sealed ampoule by Hewlett-Packard Company.

In addition, the following test mixture is used to monitor instrument performance specifically for this program:

Test Compound	Concentration (ug/ml)
Trimethyl Phosphate	20
2,6-Dimethylphenol	20
5-Cl-2-Methylaniline	20
Tri-n-butyl Phosphate	20
Dibenzothiophene	20

Solvent = Ethyl acetate or Hexane

8. PROCEDURES:

8.1 QA/QC SENSITIVITY CHECK: The performance of the chromatograph is monitored by injection of the test solution daily, before each set of samples, after the installation of new columns or detectors, or as a minimum every 20th sample. The criteria for an acceptable detector sensitivity test run are met if each compound gives a peak greater than ten times the signal/noise ratio.

8.2 ORDER OF ANALYSIS: To eliminate false positives, the instrument should be checked for sample carry-over each day by injecting in the order 1) test solution, 2) solvent blank, and 3) sample. Any suspicion of memory effects must be checked by analyzing blanks between samples. If carryover is found, the source (syringe, syringe-cleaning solution, injection port, column, etc.) must be found and the problem corrected.

8.3 QUANTITATION: Quantitation will be either by internal standard (IS) or by using a linear regression calibration curve. The internal standard may be selected from one of the test compounds that does not interfere with the compounds being analyzed. Response factors will be obtained by comparing the peak areas obtained for a solution containing a known concentration ratio of IS and analyte. For linear regression analysis, a house checkpoint solution will be prepared to check daily the validity of the calibration curve. A new curve is generated if a deviation of more than 10% is observed.

8.3 INSTRUMENT OPERATING PARAMETERS: The following are the recommended operating parameters for this program:

GC	Injector Temperature	260°C
	Interface Temperature	260°C
	Septum Flow	5 ml/min
	Carrier Gas Flow	2 ml/min
	Split Ratio	20:1
	Oven Temp Program	40°C (1 min) - 280°C (10 min) at 10°C/min

9.0 TREATMENT OF DATA: GC retention times are compared to those of authentic compounds. Results are then correlated with those obtained with other analytical methods in this program.

10.0 REFERENCE: "International Interlaboratory Comparison (Round Robin) Test for the Verification of Chemical Disarmament F.3. Testing of Procedures or Simulated Military Facility Samples", The Ministry of Foreign Affairs of Finland, Helsinki, 1992.

CHEMISTRY DEPARTMENT ANALYTICAL METHOD NO. 003.00

NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY PROCEDURES FOR THE INTERNATIONAL INTERLABORATORY TREATY VERIFICATION ROUND ROBIN EXERCISES

1. DATE: February 1993

2. PREVIOUS REVISIONS: None

3. OFFICE OF CONTACT:

Analytical Methodology Team, Chemistry Department
Research and Technology Directorate, ERDEC
SCBRD-RTS
POC: Linda L. Szafraniec or William T. Beaudry
(410) 671-3863

4. APPLICATION: NMR is used to identify and/or confirm the presence of treaty related compounds, their precursors and/or their degradation products in complex matrices. The method is applicable to all compounds dissolving in sufficient amount in the deuterated solvents used in NMR spectroscopy.

4.1 CONCENTRATION RANGE: Detection Limit to Neat

4.2 DETECTION LIMITS: The detection limit for any compound depends on the field strength of the instrument used to evaluate the sample, the accumulation time for the FT experiment, the nucleus observed, and the diameter of the NMR tube used.

Examples: ^{1}H, 400 MHz, 5-mm NMR tube, 16 hrs accumulation time (over night): 10 $\mu g/ml$ or greater.

^{19}F, 396 MHz, 5-mm NMR tube, 16 hrs accumulation time: 1 $\mu g/ml$ or greater.

^{31}P, 162 MHz, 5-mm NMR tube, 16 hrs accumulation time: 10 $\mu g/ml$ or greater.

^{13}C, 100 MHz, 5-mm NMR tube, 16 hrs accumulation time: 100 $\mu g/ml$ or greater.

^{31}P, 81 MHz, 5-mm NMR tube, 16 hrs accumulation time: 50 $\mu g/ml$ or greater.

4.3 INTERFERENCES: Any compound or compounds that have the same resonance frequency(ies) as the compound(s) of interest.

4.4 ANALYSIS RATE: 0.5 to 24 hrs/sample depending on the detectable limit desired.

4.5 VALIDATION: Compound identifications are validated by comparison to spectra in a database library or to spectra of the authentic compounds that may be acquired during these exercises. For the same solvent and the same concentration, the chemical shift values for the unknown should be within $\pm$0.1 ppm of those in the database and the coupling constants should be within $\pm$0.5 Hz. When unknown compounds (not found in the database) are present, the NMR experiments will be planned so as to obtain the structural information needed as a complement to MS, IR, and other techniques. In these cases, compound identification is validated when all of the spectroscopic data available is consistent with the proposed structure.

5.0 SCIENTIFIC BASIS: NMR spectroscopy is one of the most powerful techniques for elucidating the molecular structure of organic compounds. The sample (usually a liquid or a solid dissolved in an appropriate deuterated solvent) is placed within a strong, homogeneous magnetic field. Nuclei possessing magnetic moments within the sample align themselves with or against the direction of the static field. The sample is then irradiated with radio frequency (RF) electromagnetic radiation, causing these nuclei to resonate and flip orientation with respect to the magnetic field. This ultimately results in an NMR spectrum - a plot of resonance frequency vs peak intensity. The RF frequency used to irradiate the sample at a particular magnetic field strength dictates which nuclei will be observed in the NMR spectrum. Thus, for a magnetic field of 9.4 T, protons will be observed at 400 MHz, ^{19}F nuclei at 376 MHz, ^{31}P nuclei at 162 MHz, and ^{13}C nuclei at 100 MHz.

The three NMR parameters of interest for the Round Robin exercises are: (1) the chemical shift, which gives information about the immediate environment of the nuclei of interest; (2) the coupling constant or J value, which gives information about the number and types of neighboring magnetic nuclei; and (3) the intensity of the resonance, which is directly proportional to the number of nuclei experiencing a particular magnetic environment. With this information, supplemented by complementary MS and IR data, NMR can be used to detect and confirm the presence of treaty related CW compounds, their precursors and degradation products. Furthermore, these NMR parameters can be used to elucidate the structures of unknown organic components that may also be of interest in these exercises.

6. APPARATUS:

6.1 INSTRUMENTATION:

Varian VXR-400S Superconducting FTNMR System operating at 400 MHz for ^{1}H, 376 MHz for ^{19}F, 162 MHz for ^{31}P, and 100 MHz for ^{13}C observation.

Varian XL-200 Superconducting FTNMR System operating at 200 MHz for ^{1}H, 81 MHz for ^{31}P, and 50 MHz for ^{13}C observation.

Both spectrometers are equipped with variable temperature (VT) controllers, proton/broadband switchable VT probes, and utilize WALTZ proton decoupling during broadband operation.

6.2 HARDWARE/GLASSWARE:

New, clean, dry commercially available Pyrex NMR tubes. Tubes are inspected for scratches and nicks; if any are found, the tube is not used for CW work. Wilmad 5-mm o.d. tubes catalog nos. 507-PP, 526-PP or 527-PP may be used.

New, clean, dry glass vials with Teflon caps, glass pipettes, and syringes, as required.

Parafilm, American Can Company.

6.3 CHEMICALS:

Chloroform-d (99.8 atom % D); MD-591, Lot No. 2080-0; MSD Isotopes, Montreal, CA.

Methanol-d_4 (99.8 atom % D); Isotec, Inc., Miamisburg, OH.

Deuterium Oxide (99.9 atom % D); MD-175, Lot No. 4069-P; MSD Isotopes, Montreal CA.

Tetramethylsilane, (TMS, 99.5% pure); Norell Chemical Co., Landing NJ.

7. STANDARDS:

7.1 CALIBRATION STANDARDS: The standards for calibrating the NMR spectrometers are supplied by the manufacturer in sealed NMR tubes.

^{1}H Line Shape: 1% Chloroform, 99% Acetone-d_6

^{1}H Resolution: 1% o-Dichlorobenzene, 99% Acetone-d_6

^1H Sensitivity: 0.1% Ethyl Benzene in $CDCl_3$

^{13}C Sensitivity: 40% p-Dioxane, 60% Benzene-d_6

^{19}F Sensitivity: 0.05% $F_3C-C_6H_5$ in C_6D_6

^{31}P Sensitivity: 0.0485M Triphenyl Phosphate in $CDCl_3$

8. PROCEDURES:

8.1 EXPERIMENTAL PROCEDURE: The soil samples and the aqueous liquid samples are prepared for NMR as outlined in Schemes 1 and 2, respectively. Each sample is placed into a clean, dry 5-mm o.d. Pyrex NMR tube. The tube is capped with a pressure cap, and the top of the tube is wrapped with Parafilm. Multinuclear NMR spectra are obtained for each sample, as required.

The spectrometer is tuned, the homogeneity (i.e., resolution) adjusted, and the signal-to-noise ratio (S/N) measured using the test sample for the particular observe nucleus. The test spectrum for that nucleus is recorded, and the following criteria must be met before continuing with the unknown samples:

^1H Sensitivity	$\geq$ 100:1
^{19}F Sensitivity	$\geq$ 100:1
^{13}C Sensitivity	$\geq$ 120:1
^{31}P Sensitivity	$\geq$ 100:1

For each unknown sample, the spectrometer is tuned and the homogeneity adjusted. Data for each nucleus of interest is then accumulated until the desired signal-to-noise level has been achieved (5 min to >16 hrs). After data accumulation, the NMR spectra are stored on hard disk, and hard copies plotted out. The NMR parameters are calculated, and the spectra are compared with a database or manually interpreted by experienced NMR spectroscopists to identify the compounds present.

Both one- and two-dimensional NMR data are acquired using the standard software supplied by the manufacturer.

8.2 OPERATING PARAMETERS: The following operating parameters are recommended for this exercise:

^1H:	Operational Frequency:	400 MHz (or 200 MHz)
	Mode of Operation:	Locked, Double Precision
	Pulse Sequence:	S2PUL
	Pulse Angle:	45° or greater
	Spectral Width:	12K Hz (4K Hz)
	Data Points:	72K (30K)

	Acquisition Time:	>3 sec
	Number of Scans:	As required
	Repetition Time:	>4 sec
	Temperature:	+22 ± 1 °C
	Reference Standard:	Internal TMS, 0 ppm (organic solvents), HDO at 4.8 ppm (aqueous)
^{19}F:	Operational Frequency:	376 MHz
	Mode of Operation:	Unlocked, Double Precision
	Pulse Sequence:	S2PUL
	Proton Decoupling:	None
	Pulse Angle:	45° or greater
	Spectral Width:	100K Hz
	Data Points:	128K
	Acquisition Time:	>0.65 sec
	Number of Scans:	As required
	Repetition Time:	>2.5 sec
	Temperature:	+22 ± 1 °C
	Reference Standard:	External $CFCl_3$
^{31}P:	Operational Frequency:	162 MHz (81 MHz)
	Mode of Operation:	Unlocked, Double Precision
	Pulse Sequence:	S2PUL
	Proton Decoupling:	WALTZ, Gated On During Acquisition
	Pulse Angle:	>35°
	Spectral Width:	40K Hz (20K Hz)
	Data Points:	64K
	Acquisition Time:	0.8 sec (1.6 sec)
	Number of Scans:	As required
	Repetition Time:	>3.3 sec
	Temperature:	+22 ± 1 °C
	Reference Standard:	External 85% H_3PO_4
^{13}C:	Operational Frequency:	100 MHz (50 MHz)
	Mode Of Operation:	Locked, Double Precision
	Pulse Sequence:	S2PUL
	Proton Decoupling:	WALTZ, Full
	Pulse Angle:	>75°
	Spectral Width:	25K Hz (10K Hz)
	Data Points:	80K (32K)
	Acquisition Time:	1.6 sec
	Number of Scans:	As required
	Repetition Time:	>3.6 sec
	Temperature:	+23 ± 1 °C

Reference Standard: Internal TMS, 0 ppm (organic solvents). External TSP/D_2O, 0 ppm (aqueous)

8.3 CORRECTIVE MEASURES: If the spectrometer does not meet the manufacturer's specifications with regard to pulse widths, decoupler power, resolution, stability, lineshape and sensitivity, immediately call for emergency repair service.

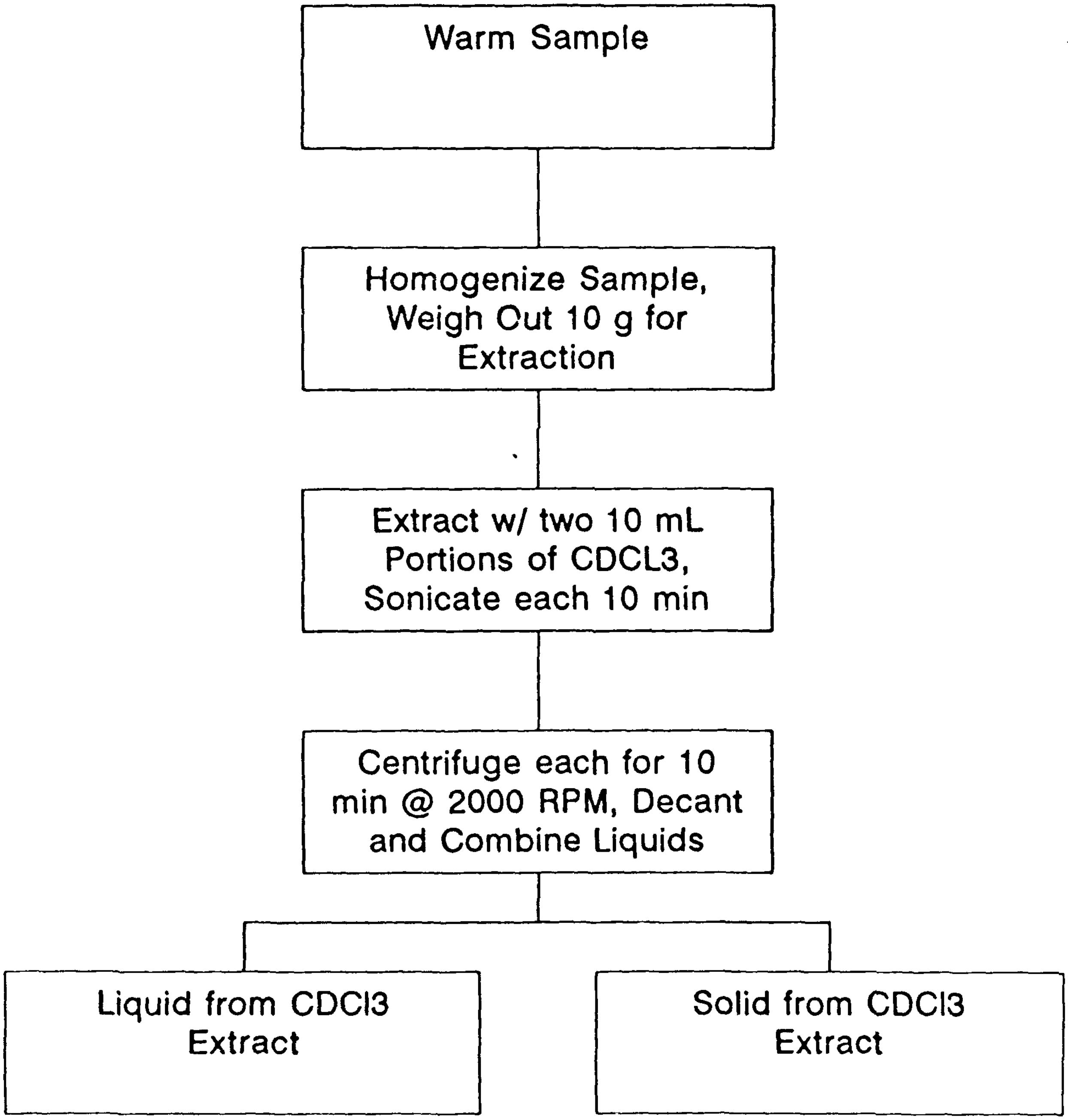
9.0 TREATMENT OF DATA: The NMR chemical shifts and coupling constants for each nucleus observed are determined and compared with spectra in the database or with spectra taken of the authentic compounds. Results are correlated with other techniques (GC/MS, IR, HPLC), and record sheets supplied for the Round Robin exercise are completed and submitted for inclusion in the final formal report.

10.0 REFERENCES:

(1) F.3. "Testing of Procedures on Simulated Military Facility Samples." International Interlaboratory Comparison (Round Robin) Test for the Verification of Chemical Disarmament. The Ministry for Foreign Affairs of Finland. Helsinki 1992.

Scheme 1

Soil Sample Preparation for NMR Analysis



Scheme 1 (Continued)

Soil Sample Preparation for NMR Analysis

Liquid from CDCl3 Extract

Dry w/ Anhydrous Sodium Sulfate

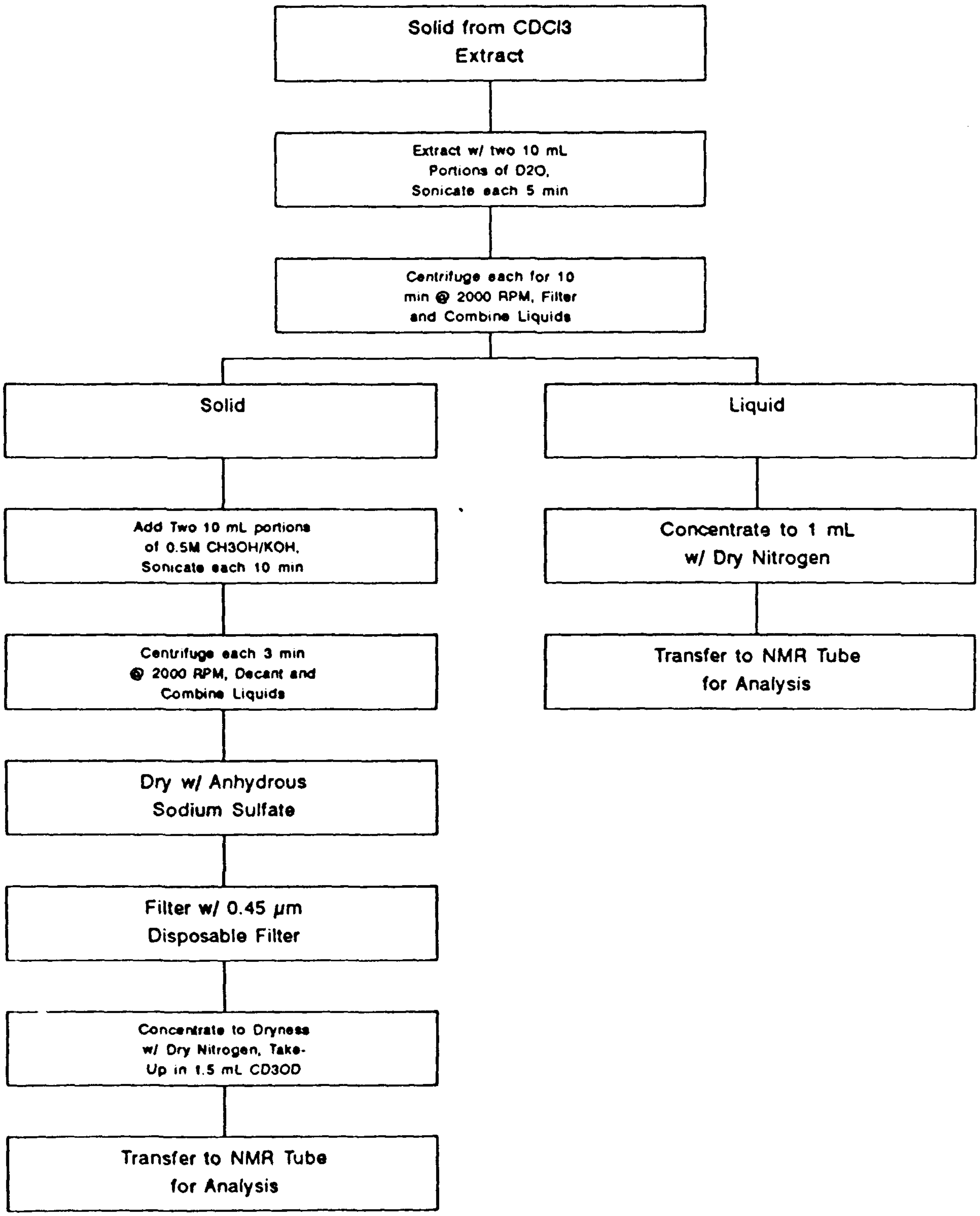
Filter thru 0.45 μm Disposable Filter

Concentrate to 1 mL w/ Dry Nitrogen

Transfer to NMR Tube for Analysis

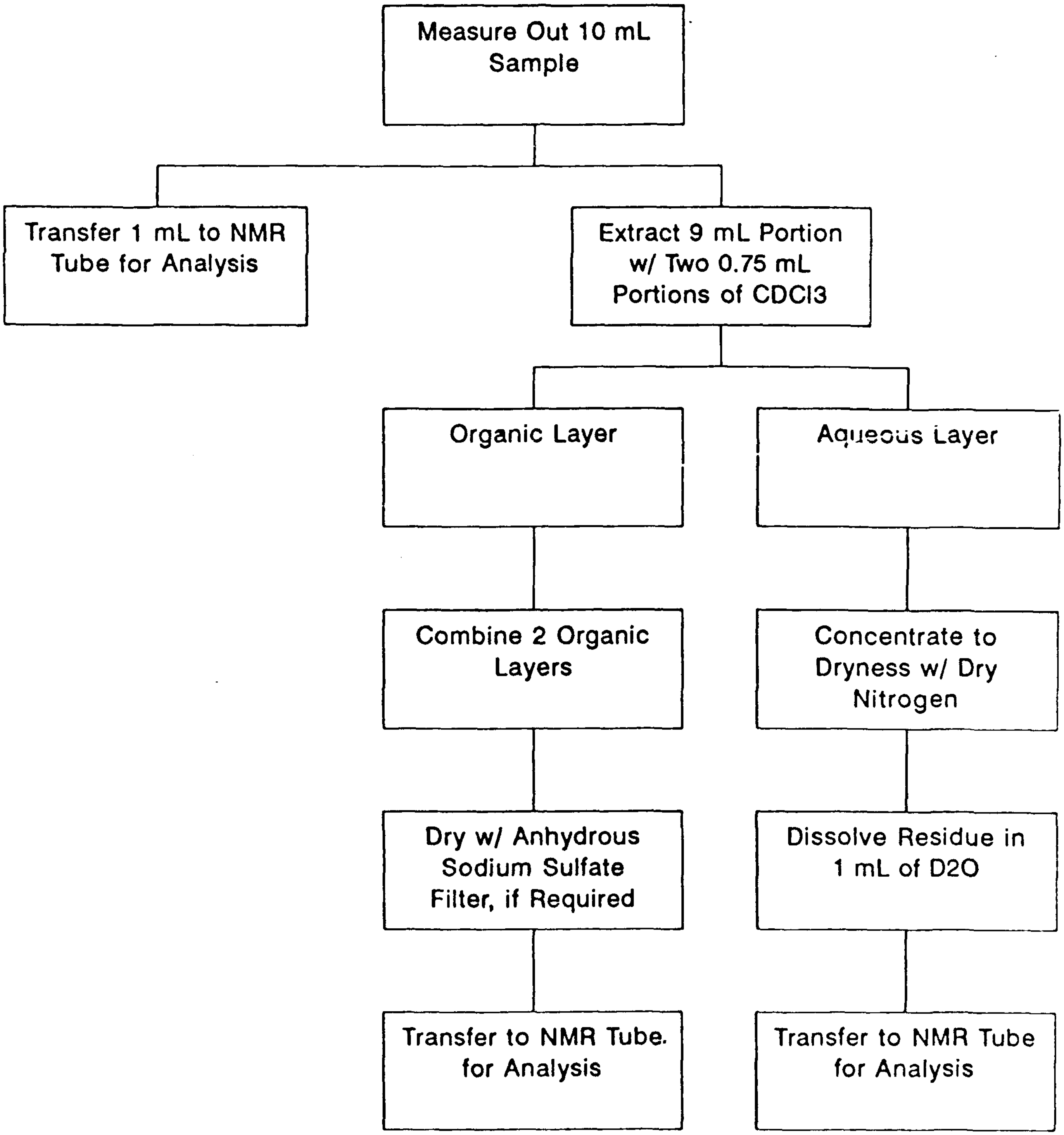
Scheme 1 (Continued)

Soil Sample Preparation for NMR Analysis



Scheme 2

Aqueous Liquid Sample Preparation for NMR Analysis



HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) PROCEDURES FOR THE INTERNATIONAL INTER-LABORATORY TREATY VERIFICATION ROUND-ROBIN EXERCISE

1. DATE: February 1993

2. PREVIOUS REVISIONS: None

3. OFFICE OF CONTACT:

Analytical Methodology Team, Chemistry Department
Research and Technology Directorate, ERDEC
SCBRD-RTC
POC: Paul C. Bossle
(410) 671-2794

4. APPLICATION: This method applies to the use of high performance liquid chromatography (HPLC) for the detection and identification of treaty-related chemical warfare (CW) scheduled compounds in complex matrices. This method applies to both ionic and neutral species that can be extracted into water. Both reverse phase liquid chromatography (RPLC), ion-exchange (ion) chromatography (IC) and ion-exclusion chromatography (ICE), are used as separation modes and ultraviolet, electrochemical, and suppressed conductivity are used as detection modes.

4.1 CONCENTRATION RANGE: Detection limits to neat.

4.2 DETECTION LIMITS: Detection limits are instrument specific and compound specific. In general, detection limits are about 40-100 ng/mL for all detection modes.

4.3 INTERFERENCES: Any compound which coelutes with the analytes of interest.

4.4 ANALYSIS RATE: One sample per 30 minutes.

4.5 VALIDATION: Compound identification are validated by comparison of HPLC retention times and detector(s) response to those of authentic samples.

5. SCIENTIFIC BASIS:

The HPLC consists of three main sections. First, the injector, where the samples are introduced normally by syringe. Then the column, where samples are separated into one or more compounds and finally, the detector(s). For example, the ultraviolet detector detects compounds by the adsorption of light on a photomultiplyer tube which in turn deflects a pen on a recorder. This deflection is a peak which denotes a compound having a specific retention time.

A HPLC is set up by following the specification of the manufacturer's manual. This information describes flow rates, optimization procedures, and use of performance test standards. The performance test standards show that the HPLC has been properly set up and is working to specification. Once the HPLC is set up, a run is made by injecting sample onto the column via the injection port. This is done by first pulling a specific amount of sample into the syringe being sure that there are no air bubbles in the syringe Next, the needle of the syringe is pushed through the septum of the injector and the plunger is pushed down. To effectively expel the entire contents of the syringes, leave the needle in the injector for 5 seconds before removing. At the time of the injection, the HPLC program and the integrator buttons are pressed.

Calibration of the HPLC is accomplished through the use of standards. These standards are compounds that are expected to be seen during a run of an unknown. A calibration curve should be run for each compound at the concentration levels expected. The calibration curve consists of five different points that vary only in concentration. Once a calibration curve is established for a specific compound, an unknown concentration of that compound can be found by using a ratio formula.

In order to ensure the calibration remains intact from day to day, a test mix of known concentration is run periodically during a day. The test mix consists of compounds which have been calibrated, then by using the area counts and retention times of the peaks in the test mix, the concentration of each compound is determined. If the determined calibration differs by +/- 10%, then the performance test mix should be run. This will indicate if the HPLC is still running at the optimal level. If this test shows a difference of more than +/- 10% then procedures for calibration should be repeated.

6. APPARATUS:

6.1 INSTRUMENTATION:

Waters Associates high performance liquid chromatograph consisting of two model 6000A pumps, a model U6K injection, model 840 data control station, a model 490 UV detector, and a model 460 electrochemical detector.

Dionex model DX 300 ion chromatograph with variable ultraviolet detector and PED electrochemical-conductivity detector.

6.2 HARDWARE/GLASSWARE: Microliter syringes

6.3 REAGENTS: Ultra high purity deionized distilled water (18 meq/cm), potassium hydrogen phosphate, perchloric acid, and HPLC grade acetonitrile.

6.4 HPLC COLUMNS: Recommended columns for this program are (1) for RPLC ODS, 5 um, 25 cm X 4.6 mm I.D.; (2) for IC, Dionex HPIC-AS4A column and (3) for ICE, Dionex HPICE-AS1 columns.

7. STANDARDS: Concentrations of calibration standards prepared in accordance with surety SOPs.

8. PROCEDURES:

8.1 INSTRUMENT OPERATING PARAMETERS: Instrument operating parameters are instrument specific. The following are the recommended operating parameters for this program:

RPLC:

Injection volume	100 uL
Eluent	90% 100 mm KH_2PO4 Buffer/10% AcCN
Column	ODS, 5 um, 25 cm X 4.6 mm I.D.
Flow Rate	1.5 mL/min
Ultraviolet detection	225 nm (0.05 AUFS)
Electrochemical detection	980 mV

Ion Chromatography (IC):

Injection volume	25 uL
Eluent	0.75 mm $NaHCO_3$/2.2 mm Na CO_3 buffer
Flow rate	0.5 mL/min
Column	Dionex HPIC-AS4A (2 mm)
Conductivity detection	3 uS sensitivity

Ion Exclusion (ICE):

Injection volume	200 uL
Eluent	100 mm perchloric acid
Flow rate	1 mL/min
Column	Dionex HPICE-AS1
Ultraviolet detection	210 nm (0.05 AUFS)
Pulsed electrochemical	E_1(0.3 V)(t60 ms), E_2(1.25 V)(t60 ms), and E_3(-0.10 V) (t240 ms)

9. TREATMENT OF DATA: HPLC retention times and detection responses are compared to those of authentic compounds. Results are then correlated with those obtained with other analytical methods in this program. Results are also compared to blank runs.

10. REFERENCE: "International Interlaboratory Comparison (Round-Robin) Test for the Verification of Chemical Disarmament F.3. Testing of Procedures on Simulated Military Facility Samples," The Ministry of Foreign Affairs of Finland, Helsinki, 1992.

CHEMISTRY DEPARTMENT ANALYTICAL METHOD NO. 005

FOURIER TRANSFORM INFRARED (FTIR) PROCEDURES FOR THE INTERNATIONAL INTERLABORATORY TREATY VERIFICATION ROUND ROBIN EXERCISES

1. DATE: February 1993

2. PREVIOUS REVISIONS: None

3. OFFICE OF CONTACT:

Sample Analysis Team, Chemistry Department
Research and Technology Directorate, ERDEC
SCBRD-RTC
P.O.C.: Thomas E. Rosso or Michael W. Ellzy
(410) 671-2790/3563

4. APPLICATION: This method applies to the use of FTIR spectroscopy for the detection and identification of treaty-related CW scheduled compounds in complex matrices. FTIR methods include solid and liquid samples, the use of light pipe for gas chromatography/infrared (GC/IR) and the use of Argon in Helium to produce matrix isolation (MI) spectra.

4.1 CONCENTRATION RANGE: Detection limit to neat

4.2 DETECTION LIMITS:

Standard IR (use of windows)	= 500 ug/mL
GC/IR from the light pipe	= 100 ug/mL
MI/IR	= 0.1 ug/mL

4.3 INTERFERENCES: For the GC systems any compound which coelutes with the analytes of interest.

4.4 ANALYSIS RATE: One sample per hour

4.5 VALIDATION: Compound identifications are validated by comparison of infrared spectra to those of authentic samples either by direct comparison or by comparison to spectra in a data base library. Operator may also validate unknown spectra by using interpretation. Systems do not necessarily complement each other. Comparison to authentic samples and confirmation by at least two separate spectrometric techniques constitutes unambiguous identification.

5.0 SCIENTIFIC BASIS: Infrared (IR) spectrometry deals with the interrelation of infrared radiation with matter. Fourier Transform IR is mainly based on the Michelson interferometer. A spectrum is obtained by measuring the absorption of IR radiation. The IR spectrum of a polyatomic molecule can be quite complex because of the many possible vibrational transitions and the existence of overtones and sum and difference bands. However, the IR spectrum for a given molecule is also unique to that molecule and thus highly useful in compound identification.

6. APPARATUS:

6.1 INSTRUMENTATION:

Nicolet 60SX FT-infrared spectrometer equipped with TGS (triglyceride sufide) detector, a Spectra-Tech IRPlan microscope and a 1 mm ID light pipe connected to a Hewlett Packard 5880 gas chromatograph.

Perkin-Elmer 1750 Model FTIR with TGS detector, sample shuttle and micropellet holder.

Mattson Cryolect 4800 Matrix Isolation Fourier Transform Infrared system with a Varian 3400 gas chromatograph inlet system. Sample can also be split to a Finnigan ITD-40 Ion trap detector giving the capability of FID/ITD/MI-FTIR with a single injection.

6.2 HARDWARE/GLASSWARE: Microliter syringes

6.3 REAGENTS: Ultrahigh purity carrier gas (Helium), compressed air is supplied by an in-house generator with dryer, hydrogen and 5% Argon in helium.

6.4 GAS CHROMATOGRAPHIC COLUMNS: Recommended column for this program is a fused silica SE-54 (DB-5), 25 m x 0.25 mm i.d., 0.25um film thickness. DB-1 and DB-1701 also give satisfactory results.

7. STANDARDS:

7.1 CALIBRATION STANDARD: Manufacturer provided test mix (i.e. polystyrene)

8. PROCEDURES:

8.1 GC INLETTING SYSTEMS CALIBRATION: The same method for calibrating the gas chromatograph is used here to ensure proper conditions of the inletting system. Separate calibrations are performed to the IR itself.

8.2 INFRARED CALIBRATION: A tune macro is run every day to show the center-burst from the interferogram and to correct the gain or mirror velocity. A 100% line will ratio a background scan versus a sample scan and the result should be a straight line +/- 0.05%. Along with the 100% line a signal-to-noise macro is run every day with an acceptable ratio of above 600:1.

8.3 INSTRUMENT OPERATING PARAMETERS: Instrument operating parameters are intrument specific. The following are the recommeded operating parameters for this program:

GC:	Injector Temperature	280 degrees C
	Detector Temperature (FID)	300 degrees C
	Carrier Gas Flow	2 mL/min.
	Split Ratio	Varies upon sample concentration
	Oven Temp. Program	60 (5 min) - 250 (5 min) at 10 degrees per min
LIGHT PIPE:	Transfer line	200 degrees C
	Light pipe	200 degrees C
	Resolution	4 wavenumber
	Scan Range	4000 - 750 wavenumbers
	Detector	MCT-A (liquid Nitrogen cooled)
MATRIX ISOLATION:		
	Transfer Line	250 degrees C
	Resolution	8 wavenumber
	Scan Range	4000 - 700 wavenumbers
	Detector	MCT-A (liquid Nitrogen cooled)
	Matrix Flow	0.3 mL/min
	Make-up Gas Flow	5 mL/min.
	Cryogenic Disk:	
	Temperature	10 degrees Kelvin
	Pressure	3 x 10-5 Torr

Blank

APPENDIX I

SUMMARY OF QUANTITATIVE RESULTS

Table I-1. Summary of Quantitative Results - Water Sample W-15

Compound Number	Compound Name	Total amount [μg/sample]	Analytical Method*
1	Methylphosphonic Acid	450	^{1}H NMR
1	Methylphosphonic Acid	500	LC1
1	Methylphosphonic Acid	578[a]	GC2/MS2
1	Methylphosphonic Acid	174[a]	GC3/MS3/SIM
2	2-Diisopropylaminoethanol	400	^{1}H NMR
2	2-Diisopropylaminoethanol	266	GC4
2	2-Diisopropylaminoethanol	308	GC2/MS2
2	2-Diisopropylaminoethanol	152	GC3/MS3/SIM
3	3-Quinuclidinol	226	GC4
3	3-Quinuclidinol	900	^{1}H NMR
3	3-Quinuclidinol	1038[a]	GC1/MS1
3	3-Quinuclidinol	1113[a]	GC2/MS2
3	3-Quinuclidinol	544[a]	GC3/MS3/SIM
4	Benzilic Acid	400	^{1}H NMR
4	Benzilic Acid	500	LC1
4	Benzilic Acid	370	GC4
4	Benzilic Acid	549	GC2/MS2
4	Benzilic Acid	171	GC3/MS3/SIM

[a] BSTFA derivatization of acidified 1 mL sample, all others according to ROP.

* Method numbers are assigned in Appendixes E, F and G.

Table I-2. Summary of Quantitative Results - Water Sample KEW-14

Compound Number	Compound Name	Total amount [μg/sample]	Analytical Method*
1	Methylphosphonic Acid	450	^1H NMR
1	Methylphosphonic Acid	500	LC1
1	Methylphosphonic Acid	83	GC4
1	Methylphosphonic Acid	14[a]	GC3/MS3/SIM
2	2-Diisopropylaminoethanol	50	^1H NMR
2	2-Diisopropylaminoethanol	60	GC4
2	2-Diisopropylaminoethanol	179	GC2/MS2
2	2-Diisopropylaminoethanol	58	GC3/MS3/SIM
3	3-Quinuclidinol	650	^1H NMR
3	3-Quinuclidinol	431	GC4
3	3-Quinuclidinol	212[b]	GC2/MS2
3	3-Quinuclidinol	215[a]	GC3/MS3/SIM
4	Benzilic Acid	350	^1H NMR
4	Benzilic Acid	150	LC1
4	Benzilic Acid	276	GC4
4	Benzilic Acid	177	GC2/MS2
4	Benzilic Acid	132	GC3/MS3/SIM

[a] Obtained with BSTFA derivative of acidified 1 mL sample.
[b] Obtained with BSTFA derivative of NMR extract KEW-14C.

* Method numbers are assigned in Appendix E, F and G.

Table I-3. Summary of Quantitative Results - Cartridge Sample K-15

Compound Number	Compound Name	Total amount [μg/sample]	Analytical Method*
2	2-Diisopropylaminoethanol	6	GC2/MS4
2	2-Diisopropylaminoethanol	12	GC3/MS3/SIM
3	3-Quinuclidinol	4	GC3/MS3/SIM
4	Benzilic Acid	41	GC2/MS4
4	Benzilic Acid	177	GC4
4	Benzilic Acid	48	GC3/MS3/SIM

* Method numbers are assigned in Appendixes E, F and G.

Table I-4. Summary of Quantitative Results - Soil Sample SN-15

Compound Number	Compound Name	Total amount [μg/sample]	Analytical Method*
1	Methylphosphonic Acid	200	LC1
1	Methylphosphonic Acid	283	GC2/MS4
1	Methylphosphonic Acid	239[a]	GC3/MS3/SIM
2	2-Diisopropylaminoethanol	317	GC2/MS4
2	2-Diisopropylaminoethanol	32	GC3/MS3/SIM
3	3-Quinuclidinol	461	GC1/MS1
3	3-Quinuclidinol	443	GC3/MS3/SIM
4	Benzilic Acid	578	GC1/MS1
4	Benzilic Acid	155	GC2/MS2
4	Benzilic Acid	200	GC4
4	Benzilic Acid	200	LC1
4	Benzilic Acid	351[a]	GC3/MS3/SIM
[a] Obtained after second diazomethane derivatization.			

* Method numbers are assigned in Appendixes E, F and G.

Table I-5. Summary of Quantitative Results - Soil Sample SA-15

Compound Number	Compound Name	Total amount [μg/sample]	Analytical Method*
1	Methylphosphonic Acid	400	LC1
1	Methylphosphonic Acid	5[a]	GC3/MS3/SIM
2	2-Diisopropylaminoethanol	25[a]	GC3/MS3/SIM
3	3-Quinuclidinol	5[a]	GC3/MS3/SIM
4	Benzilic Acid	100	LC1
4	Benzilic Acid	84[a]	GC3/MS3/SIM
[a] Soil and water pH adjusted to 7, sonicated 40 min, pH readjusted to 7, sonicated 10 min, allowed to sit overnight, filtered, dried, and derivatized with BSTFA.			

* Method numbers are assigned in Appendixes E, F and G.

Table I-6. Summary of Quantitative Results - Soil Sample SB-15

Compound Number	Compound Name	Total amount [μg/sample]	Analytical Method*
1	Methylphosphonic Acid	300	LC1
1	Methylphosphonic Acid	10[a]	GC3/MS3/SIM
2	2-Diisopropylaminoethanol	21	GC3/MS3/SIM
3	3-Quinuclidinol	402	GC1/MS1
3	3-Quinuclidinol	442	GC3/MS3/SIM
4	Benzilic Acid	164	GC1/MS1
4	Benzilic Acid	236	GC2/MS2
4	Benzilic Acid	220	GC4
4	Benzilic Acid	200	LC1
4	Benzilic Acid	248	GC3/MS3/SIM
[a] Obtained after second diazomethane derivatization.			

* Method numbers are assigned in Appendixes E, F and G.

APPENDIX J

SUMMARY OF ANALYTICAL METHODS

Table J-1. Summary of Analytical Methods - Water Sample W-15

Compound Number	Compound Name	Unambiguous/ Tentative[a]	Derivative	Analytical Methods[b]
1	Methylphosphonic Acid	U	None	^{1}H NMR; ^{31}P NMR ^{13}C NMR; LC1
			TMS	GC1/MS1; GC2/MS2 GC3/MS3/SIM
2	2-Diisopropylaminoethanol	U	None	^{1}H NMR; GC1/MS1 GC2/MS2; GC4 GC3/MS3/SIM
			TMS	GC1/MS1; GC2/MS2 GC3/MS3
3	3-Quinuclidinol	U	None	^{1}H NMR; ^{13}C NMR GC4
			TMS	GC1/MS1; GC2/MS2 GC3/MS3
4	Benzilic Acid	U	None	^{1}H NMR; ^{13}C NMR LC1
			Methyl	GC1/MS1; GC2/MS2 GC3/MS3; GC4
			TMS	GC1/MS1; GC2/MS2 GC3/MS3

[a] U = Unambiguous Identification; T = Tentative Identification
[b] See Appropriate Appendix For Description of Methods.

Table J-2. Summary of Analytical Methods - Water Sample KEW-14

Compound Number	Compound Name	Unambiguous/ Tentative[a]	Derivative	Analytical Methods[b]
1	Methylphosphonic Acid	U	None	^{1}H NMR; ^{31}P NMR LC1
			Methyl	GC4
			TMS	GC3/MS3/SIM
2	2-Diisopropylaminoethanol	U	None	^{1}H NMR; GC1/MS1 GC2/MS2; GC2/MS4 GC4; GC3/MS3/SIM
			TMS	GC3/MS3/SIM
3	3-Quinuclidinol	U	None	^{1}H NMR; GC4
			TMS	GC2/MS2; GC3/MS3
4	Benzilic Acid	U	None	^{1}H NMR; LC1
			Methyl	GC1/MS1; GC2/MS2 GC3/MS3; GC4
			TMS	GC2/MS2 GC3/MS3/SIM

[a] U = Unambiguous Identification; T = Tentative Identification
[b] See Appropriate Appendix For Description of Methods.

Table J-3. Summary of Analytical Methods - C18 Cartridge Sample K-15

Compound Number	Compound Name	Unambiguous/ Tentative[a]	Derivative	Analytical Methods[b]
2	2-Diisopropylaminoethanol	U	None	GC2/MS4 GC3/MS3/SIM
3	3-Quinuclidinol	T	TMS	GC3/MS3/SIM
4	Benzilic Acid	U	Methyl	GC2/MS4; GC4 GC3/MS3/SIM

Table J-4. Summary of Analytical Methods - Soil Sample SA-15

Compound Number	Compound Name	Unambiguous/ Tentative[a]	Derivative	Analytical Methods[b]
1	Methylphosphonic Acid	T	None	^{31}P NMR; LC1
			TMS	GC3/MS3/SIM
2	2-Diisopropylaminoethanol	U	TMS	GC1/MS1 GC3/MS3/SIM
3	3-Quinuclidinol	T	TMS	GC3/MS3/SIM
4	Benzilic Acid	U	None	LC1
			TMS	GC1/MS1 GC3/MS3/SIM

[a] U = Unambiguous Identification; T = Tentative Identification
[b] See Appropriate Appendix For Description of Methods.

Table J-5. Summary of Analytical Methods - Soil Sample SB-15

Compound Number	Compound Name	Unambiguous/ Tentative[a]	Derivative	Analytical Methods[b]
1	Methylphosphonic Acid	U	None	^{1}H NMR; ^{31}P NMR LC1
			Methyl	GC3/MS3/SIM
2	2-Diisopropylaminoethanol	U	None	^{1}H NMR GC3/MS3/SIM
			TMS	GC3/MS3/SIM
3	3-Quinuclidinol	U	None	^{1}H NMR
			TMS	GC1/MS1; GC7/MS3
4	Benzilic Acid	U	None	^{1}H NMR; LC1
			Methyl	GC1/MS1; GC2/MS2 GC3/MS3; GC4
			TMS	GC1/MS1; GC7/MS3

[a] U = Unambiguous Identification; T = Tentative Identification
[b] See Appropriate Appendix For Description of Methods.

Table J-6. Summary of Analytical Methods - Soil Sample SN-15

Compound Number	Compound Name	Unambiguous/ Tentative[a]	Derivative	Analytical Methods[b]
1	Methylphosphonic Acid	U	None	^{31}P NMR; LC1
			Methyl	GC1/MS1; GC2/MS2 GC2/MS4 GC3/MS3/SIM
			TMS	GC1/MS1; GC3/MS3
2	2-Diisopropylaminoethanol	U	None	GC2/MS2; GC2/MS4 GC3/MS3/SIM
			TMS	GC3/MS3/SIM
3	3-Quinuclidinol	U	TMS	GC1/MS1; GC3/MS3
4	Benzilic Acid	U	None	^{1}H NMR (T), LC1
			Methyl	GC1/MS1; GC2/MS2 GC3/MS3; GC2/MS4 GC4
			TMS	GC1/MS1

[a] U = Unambiguous Identification; T = Tentative Identification
[b] See Appropriate Appendix For Description of Methods.